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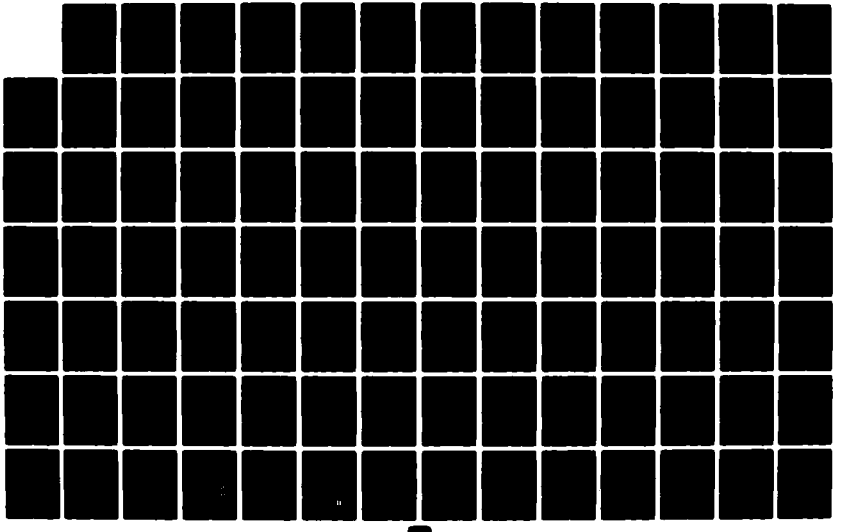

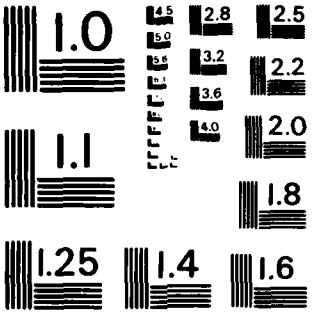

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REPORT MEDDH-288(R1) AD_____

US ARMY INSTITUTE OF SURGICAL RESEARCH ANNUAL RESEARCH PROGRESS REPORT FY 1982

U.S. ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

(1 October 1981 - 30 September 1982)

1 October 1982

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Prepared for:
US ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND
FOR DETRICK, FREDERICK, MD 21701

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

This report documents the clinical and laboratory activities of the US Army Institute of Surgical Research during the fiscal year 1982. These activities include patient care, clinical investigation and laboratory research in the areas of burn injury and general trauma. Special emphasis is placed on the clinical management of burned patients and on studies related to prevention, diagnosis and treatment of infections in severely burned patients.

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US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON, TEXAS 78234

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SUBJECT: Annual Research Report FY 1982

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Annual report(s) of the US Army Institute of Surgical Research for FY-82 are forwarded under provisions of the OTSG Regulation 70-31, dated 2 April 1969.

Basil A. Pruitt Jr.

BASIL A. PRUITT, JR, MD, FACS
COLONEL, MC
COMMANDER & DIRECTOR

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FOREWORD

The military events of the past year have served to reemphasize the importance of burns as a combat injury. In the Falkland Islands war, 18 per cent of all casualties sustained burns and in the Lebanon conflict 8.6 per cent of the injured had burn injuries. Such verification of the military relevance of the clinical care and research activities of this Institute fully justifying not only continued but expanded support thereof comes at a time when the surgical staffing of the Institute is critically low. During the current fiscal year the surgical staff will reach a nadir of three assigned individuals to accomplish a workload justifying 10 authorized positions for surgeons. The potential impact of the shortage of surgical staff has been further emphasized in the report of the civilian committee which reviewed quality of care at the Institute this year. That committee identified and highlighted the peril in which the shortage of surgeons places research activities. The 30 per cent staffing level noted above will necessitate suspension of research projects and fulfill the most dire prophecies of the site visit team. Diminution of research activity and loss of research productivity will not only abrogate further progress in the care of the combat injured soldier but lead to forfeiture of the leadership position in burn care occupied by the US Army Medical Corps for the past 36 years.

Among the factors contributing to this surgical staff crisis are a pay scale that is non-competitive with academic salaries, a perceived promotion handicap for surgeons in an R&D assignment, and a

general disdain within the Medical Corps for academically oriented scholarly activity. These disincentives must be overcome and the Institute must maintain its close contacts with academic surgical programs in order to compete successfully for the type of surgeon investigators who have been responsible for the Institute's contributions to surgical care. By identifying clinically significant problems and then collaborating with other Institute scientists to bring about solutions to those problems, our surgical staff members have played a key role in the research results reported in this volume and are necessary for the continuity of such research.

The future of this Institute and its credibility in the field of trauma surgery depend upon a surgical staff of sufficient size to ensure continued excellence of care and concomitant investigative activity. Moreover, a continuing commitment to research and academic endeavor on the part of those surgeons is essential to ensure further progress and improvement in the care of the combat injured soldier.

BASIL A. PRUITT, JR., MD, FACS
Colonel, MC
Commander and Director

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AF	164
b. CONTRIBUTING				
c. XXXXXXX	STOG 80 - 7.2:5			

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(U) Clinical Operation, Center for Treatment of Burned Soldiers

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003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
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ASSOCIATE INVESTIGATORS

NAME: Basil A. Pruitt, Jr., MD, COL, MC

NAME: POC: DA

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22. KEYWORDS (Procode EACH with Security Classification Code) (U) Heterograft; (U) Resuscitation; (U) Homograft; (U) Air Evacuation; (U) Thermal Injury; (U) Inhalation Injury; (U) Topical Therapy; (U) Autograft

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procode text of each with Security Classification Code.)

23. (U) The Clinical Division of the US Army Institute of Surgical Research continues as a major specialized clinical treatment center for thermally injured military personnel and other eligible beneficiaries. The objectives are in addition to clinical care, investigation of new diagnostic and therapeutic technics and the promulgation of scientific advances to health professionals.

24. (U) Thermal, chemical and electric injured patients from the Continental United States and throughout the world are transported to the U.S. Army Institute of Surgical Research for intensive, specialized inpatient treatment. Carefully controlled evaluation of new treatment technics is conducted by the professional staff.

25. (U) 8110 - 8209. Two hundred twenty nine seriously burned patients were admitted and treated during 1981. Active clinical research activities included investigation of the metabolic response to and nutritional support of acutely injured patients; assessment of excision and other wound care technics; the effect of colloid containing resuscitation fluid on pulmonary extravascular lung water; and the alterations of endocrine inter-relationships following injury.

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ANNUAL PROGRESS REPORT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

REPORT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 January 1981 - 31 December 1981

Investigators:

William F. McManus, MD, Colonel, MC
Anton J. Jirka, MD, Colonel, MC
Cleon W. Goodwin, MD
Esber H. Mansour, MD, Major, MC
Khan Z. Shirani, MD, Major, MC
Roosevelt J. Stallings, MD, Major, MC
George Vaughan, MD, Major, MC
Roger W. Yurt, MD, Major, MC
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Basil A. Pruitt, Jr., MD, Colonel, MC

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ABSTRACT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

REPORT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 January - 31 December 1981

Investigators: William F. McManus, MD, Colonel, MC
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During calendar year 1981, 229 patients were admitted to the Clinical Division of the United States Army Institute of Surgical Research. Principle activities of the Clinical Division included care of the severely injured patient, research to improve survival and function of the injured patient, and the education and training of health care professional and para professional personnel. The areas of research included evaluation of wound care techniques to include subeschar antibiotic administration for prevention and treatment of burn wound infection, the metabolic response to and nutritional support of the severely injured patient, the effect of colloid administration on extravascular lung water, neuro-endocrine inter-relationships and alterations following injury, and the effect of early excision of the burn wound.

Autograft
Allograft
Zenograft
Thermal Injury
Topical Therapy
Resuscitation
Aeromedical Transfer
Inhalation Injury

CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS

The Clinical Division, U.S. Army Institute of Surgical Research admitted 229 soldiers and other authorized patients with thermal, chemical, or electric injuries during calendar year 1981. Aeromedical teams from this Institute conducted 71 missions to transfer 97 (42.3%) of the 229 admitted patients. Seventy of the 71 missions were within the Continental United States for 86 patients of which 26 missions were by rotary wing aircraft and 44 were by fixed wing aircraft. In addition one OCONUS flight to Honduras was accomplished for 11 patients. Forty patients (17.5%) of the 229 admissions were admitted directly from Emergency Medical Services in the San Antonio area and not transferred from another medical facility. Seventy six (36.5%) of the 229 patients were admitted within 24 hours of injury and 127 (55.5%) were admitted within 48 hours of injury. The following statistics are based on 208 patient dispositions during calendar year 1981 of which 161 were male patients and 47 were female. The ages of these 208 patients ranged from three months to 87 years with an average of 28 years. Burn size averaged 30.3% of the body surface with an average full thickness burn of 14.7%. Thirty seven patients were in the pediatric age group (age 15 and under) with an average age of 3.8 years and an average burn size of 24.6% of the body surface. The average hospital stay was 43.6 days when convalescent leave was included in the calculation and 41.1 days when convalescent leave was subtracted. There were 17 patients with high voltage electric injury, 4 patients with chemical injury and one patient with frostbite associated with burn injury. The source of admission is identified in Table 1 and the cause of burn injury is delineated in Table 2.

MORBIDITY AND MORTALITY

Forty-three of 208 patients (20.7%) died during calendar year 1981. Autopsies were performed in 23 (53.5%) of these hospital deaths. The average burn size of patients who died was 62.2% and the full thickness average was 39.8%. The ages of patients who died ranged from 16 months to 87 years with an average age of 34.4 years. Fifteen of the 43 patients (35%) had inhalation injury as a primary diagnosis an antecedent to pneumonia as a cause of death. Seven (16%) patients died with an acute myocardial infarction. Eight patients (18.6%) had burn injury ranging from 92% to 100% of the body surface. Two patients had pulmonary emboli as a cause of death. Seven children (16.3% of deaths) died with an average total body surface burn of 58% and an average full thickness burn of 38.6%. The average age of children who died was 3.4 years (range 16 months to 6 years) and two of these seven had autopsies. Infection, again this year, was the most common

complication following injury. Fifty one of 208 patients had bacteria recovered from the blood; Pseudomonas aeruginosa in 14 patients, Staphylococcus aureus in 14 patients, Providencia stuartii in nine, Klebsiella spp. in 4 patients, and a variety of predominately gram negative organisms in the remaining ten patients. Burn wound sepsis was diagnosed in 28 patients and suppurative thrombophlebitis in five patients.

One patient required celiotomy and cecostomy for acute dilatation of the right colon and cecum. Eleven patients had clinical upper gastrointestinal hemorrhage and all responded to nonoperative therapy.

Twenty four patients had acute renal failure and seven were dialized (5 hemodialyses and 2 peritoneal dialyses). Acute myocardial infarction was diagnosed in 12 patients, 5.8% of dispositions. Bronchopneumonia was diagnosed in 52 patients, inhalation injury in 64 patients (30.8% of dispositions) and pulmonary emboli in 10 patients. Ninety-one patients (43.8%) had some associated injury (includes 64 patients with inhalation injury); fractures or dislocations in 13 patients, lacerations in 11 patients and closed head injury in nine patients were the most common associated injuries.

EDUCATION

The professional staff of the Clinical Division of the U.S. Army Institute of Surgical Research continued to be committed to providing education to all professional and paraprofessional levels locally, nationally and internationally during 1981. A total of 19 resident physicians were attached for periods of one to two months during 1981 including 5 from Fitzsimons Army Medical Center, 4 from Travis AFB, 2 from Brooke AMC, 1 each from Letterman AMC and Walter Reed AMC and 4 from civilian residency programs including 3 from William Beaumont Hospital in Royal Oak, Michigan and 1 from the University of Texas Health Science Center at San Antonio. A total of 14 medical students rotated at this center including University of Texas Southwest Medical School at Dallas, University of Chicago, Vanderbilt University, Baylor University, Albert Einstein, Rutgers and Creighton University of which 3 of these students were Health Profession Scholarship students. Physicians visited this Institute from foreign countries for periods of time ranging from 1 day to 6 months and included 24 from the Peoples Republic of China, 5 from Norway, 4 from Mexico and 1 each from Italy, Hungary, Nepal, Australia, Canada, England, Panama, Jordan, Thailand, Egypt, and Puerto Rico. The Physical Therapy Branch of the Institute had 39 trainees, both military and civilian and the Occupational Therapy Branch had 64 trainees in the calendar year 1981. Seven scientific publications appeared in refereed medical journals and approximately 150 scientific presentations

were conducted for military and civilian audiences. Numerous scientific presentations were made at the Academy of Health Sciences and various military installations throughout the Continental United States to include support of the Battlefield Medicine Course of the U.S. Air Force and the Combat Casualty Courses of the U.S. Army. In addition, weekly professional staff conferences were conducted for and by the Institute personnel.

STATISTICAL RESUME

During calendar year 1981, 229 patients were admitted to the Institute of Surgical Research and there were 208 dispositions during the same period. All subsequent data are based on dispositions. There were 161 males and 47 females with an average age of 28 years ranging from 3 months to 87 years of age. Thirty seven patients (17.8%) were less than 15 years old and 43 patients (20.6%) were over 45 years of age. The average total burn of the entire population was 30.3% of the total body surface with 14.7% average extent of full thickness injury. The average hospital stay of all patient excluding convalescent leave for active duty military was 43.6 days. One hundred twenty seven patients (62%) were admitted within 48 hours of injury.

During 1981, 1,754 operative procedures were performed on 176 patients for an average of 8 operative procedures per patient. Four hundred two anesthetics were given to 129 patients (1.9 per patient). One hundred twenty seven patients received a total of 514,000 cc of blood (4047 cc per patient).

Table 1 identifies the source of admission of patients during the calendar year 1981; Table 2 summarizes burn etiology; Table 3 lists the effective age and extent of injury on survival; and, Table 4 lists mortality rate associated with increments of 10% total body surface burn for the years 1978 through 1981. Table 5 summarizes the survival of patients with extensive burns from 1958 through 1981 and Table 6 compares mortality before and after the use of topical chemotherapy of the burn wound. Table 7 lists the cause of death for calendar year 1981.

Table 1. Source of Admission, 1981

Area	A	AD	AF	AFD	N	ND	VAB	Other	Total
1st Army	4	0	0	0	1	0	0	0	5
3rd Army	3	2	0	1	3	1	4	13	27
5th Army	17	12	9	9	3	1	12	66	129
6th Army	6	1	0	3	2	0	1	2	15
Korea	2	2	3	0	0	0	0	1	8
Germany	3	1	3	1	0	0	0	0	8
Mexico	1	0	0	0	0	0	0	0	1
Brazil	0	0	0	0	0	0	0	1	1
Panama	1	0	0	0	0	0	0	0	1
Hawaii	0	0	0	0	0	0	0	1	1
Azores	0	0	1	0	0	0	0	0	1
Honduras	0	0	0	0	0	0	0	11	11
	37	18	16	14	9	2	17	95	208

A - Army
AF - Air Force
D - Dependent
N - Navy, Marine Corps & US Coast Guard
VAB - Veterans Administration Beneficiary
Other: Civilian Emergency
US Public Health Service Beneficiary
Bureau of Employees Compensation Beneficiary

Table 2. Burn Etiology, 1981 - 208 Dispositions

Causes	Number of Patients	Disposition	Deaths	Mortality
Gasoline, Diesel & Kerosene	48	23.0%	9	18.8%
Structural Fires	28	13.5%	10	35.7%
Motor Vehicle Accidents	9	4.3%	3	33.3%
Aircraft Accidents	3	1.4%	0	0.0%
Open Flames	14	6.7%	6	42.9%
Electrical	16	7.7%	0	0.0%
Hot Liquids	31	14.9%	3	9.7%
Chemical	3	1.4%	0	0.0%
Butane, Propane or Natural, Sewer Gas Exp.	25	12.0%	10	40.0%
Welding	2	1.0%	0	0.0%
Smoking Clothes Ignited	5	2.4%	2	40.0%
Bomb, Shell, Simulator Grenade, Gunpowder Exp.	11	5.3%	0	0.0%
Others	9	4.3%	0	0.0%
Contact	4	1.9%	0	0.0%
TOTAL	208		43	

Table 3. Age, Body Surface Involvement & Mortality, 1981

Age (Yrs)	Per Cent Burn										Total Cases	Total Deaths	% Mortality
	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100			
0-1	1	1	0	1	0	0	0	0	0	0	3	0	0.0
1-2	1	3	3(1)	2	0	0	0	0	0	0	9	1	11.1
2-3	3	1	1	1	0	3(2)	0	0	0	0	9	2	22.2
3-4	2	1	0	0	0	2(2)	0	0	0	0	5	2	40.0
4-5	1	0	0	0	0	0	0	0	0	0	1	0	0.0
5-10	2	0	3	0	0	0	0	1(1)	0	1(1)	7	2	28.6
10-15	2	0	0	0	1	0	0	0	0	0	3	0	0.0
15-20	7	6	4	2	4	1(1)	0	2(2)	1	1(1)	28	4	14.3
20-30	17	10	6	13	3	9(4)	1(1)	1(1)	2(1)	4(4)	66	11	16.7
30-40	6	6	2	7	2	5(3)	2(1)	1(1)	0	0	31	5	16.1
40-50	3	3	3(1)	0	4(2)	1	0	1(1)	0	0	15	4	26.7
50-60	4	4	1(1)	1(1)	2(1)	3(1)	0	0	0	0	15	4	26.7
60-70	2	2	1	1(1)	2(1)	2(2)	0	0	0	1(1)	11	5	45.5
70-80	0	2	0	0	0	0	0	0	0	1(1)	3	1	33.3
80-90	0	0	0	1(1)	0	0	0	1(1)	0	0	2	2	100.0
90-100	0	0	0	0	0	0	0	0	0	0	0	0	0.0
Total	51	39	24	29	18	26	3	7	3	8	208		
Deaths	0	1	3	3	4	15	2	7	1	8		43	
% Mortality	0	0	12.5	10.3	22.2	57.9	66.7	100	33.3	100			20.7

Table 4. Per Cent Body Surface Burn Involvement and Mortality, 1978 - 1981

% Burn	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	TOTAL
					(1978)						
No. Burned	48	49	46	37	27	17	20	12	6	6	268
Deaths	0	4	6	10	9	8	12	12	2	6	69
% Mortality	0	8.2	13.0	27.0	33.3	47.0	60.0	100	33.3	100	25.8
					(1979)						
No. Burned	50	49	35	23	32	29	16	10	15	8	267
Deaths	0	2	0	8	8	11	12	10	15	8	74
% Mortality	0	4.0	0	34.8	25.9	37.9	75.0	100	100	100	27.7
					(1980)						
No. Burned	34	42	41	37	18	24	16	11	13	6	242
Deaths	0	1	2	3	8	12	12	11	11	6	66
% Mortality	0	2.4	4.9	8.19	44.4	50	75	100	84.6	100	27.3
					(1981)						
No. Burned	51	39	24	29	18	26	3	7	3	8	208
Deaths	0	0	3	3	4	15	2	7	1	8	43
% Mortality	0	0	12.5	10.3	22.2	57.7	66.7	100	33.3	100	20.7

Table 5. Survival and Death by Year for Patients With Extensive Burns, 1959-1981

Year	Survivors (burns over 30%)			Deaths		
	No. Cases	Average % Burn		No. Cases	Average % Burn	
		Total	3°		Total	3°
1959	29	43.1	20.6	24	63.1	38.1
1960	17	44.2	20.1	30	57.8	37.3
1961	18	44.2	25.0	31	58.0	39.7
1962	18	42.7	21.4	54	59.1	46.2
1963	28	45.8	19.6	57	69.0	41.0
1964	40	41.8	14.8	37	65.0	42.4
1965	47	43.8	21.0	33	66.0	33.4
1966	68	41.5	14.9	59	59.9	31.3
1967	103	42.7	13.3	51	59.9	32.3
1968	143	44.2	12.6	38	54.6	24.6
1969	113	43.2	11.1	70	58.7	26.4
1970	92	39.4	10.7	70	51.9	32.6
1971	63	41.9	14.0	68	60.8	38.0
1972	62	42.0	17.2	103	56.7	35.9
1973	47	43.7	19.6	113	60.3	36.2
1974	55	43.9	12.2	97	60.8	35.9
1975	80	46.1	14.7	94	61.3	32.8
1976	69	45.5	15.0	79	64.2	31.1
1977	66	42.2	14.4	70	56.9	29.0
1978	67	45.7	14.8	69	55.2	33.0
1979	61	45.4	13.4	74	65.0	37.0
1980	62	42.7	15.1	66	64.3	41.8
1981	54	42.7	17.5	43	62.2	39.8

Table 6. Comparison of Burn Mortality Rates, 1962-1963 and 1964-1981

Years	Per Cent Burn														
	0-30			30-40			40-50			50-60			60-100		
	No. Pts.	No. Deaths	% Mortality	No. Pts.	No. Deaths	% Mortality	No. Pts.	No. Deaths	% Mortality	No. Pts.	No. Deaths	% Mortality	No. Pts.	No. Deaths	% Mortality
1962-63	140	6	4.3	36	16	44.4	36	22	61.1	23	18	78.3	55	49	89.1
1964-81	2416	79	3.3	707	129	18.3	573	182	31.8	418	207	49.5	750	637	84.9

Table 7. Cause of Death, 1981

Patient	Age	Sex	% Burn Total	% Burn 3°	PEB Death	Cause of Death
1	62	M	100	92	0	*100% total body surface burn
2	16	M	99	88	1	*99% total body surface burn and severe inhalation injury
3	6	M	96.5	96.5	0	*96.5% total body surface burn and severe inhalation injury
4	25	F	96	84	9	*96% total body surface burn with Providencia septicemia
5	21	M	93	93	1	*93% total body surface burn
6	72	M	92	89	0	*92% total body surface burn
7	27	F	92	86	4	*92% total body surface burn
8	26	M	92	75	6	92% total body surface burn with myocardial necrosis
9	22	M	83.5	43	8	83.5% total body surface burn with Pseudomonas spp. pneumonia and septicemia
10	24	M	78	59.5	18	78% total body surface burn with fungal burn wound invasion and septicemia
11	46	M	77.5	22	9	77.5% total body surface burn plus acute myocardial infarction
12	18	M	77	39	23	*77% total body surface burn plus pneumonitis, septicemia and acute renal failure
13	5	F	74	74	2	74% total body surface burn plus severe inhalation injury
14	82	F	74	71	5	*74% total body surface burn plus severe inhalation injury

* Autopsy not performed

Table 7. Cause of Death, 1981 - Continued

Patient	Age	Sex	% Burn Total	% Burn 3°	PEB Death	Cause of Death
15	16	M	70	13	14	70% total body surface burn, severe inhalation injury, pneumonia and septicemia
16	33	M	70	0	20	70% total body surface burn, severe pneumonia, septicemia and acute renal failure
17	26	M	68.5	46.5	30	68.5% total body surface burn, pulmonary embolus
18	36	F	65.5	43	7	65.5% total body surface burn, inhalation injury and pneumonia
19	25	M	59	53	16	53% total body surface burn and pneumonia
20	58	M	59	25	10	*59% total body surface burn, inhalation injury and pneumonia
21	61	M	58.5	2.5	14	Acute myocardial infarction
22	16	F	56.5	24.5	26	*56.5% total body surface burn, plus invasive burn wound sepsis
23	34	F	56.5	7	67	Acute myocardial infarction
24	27	M	56	55	7	Inhalation injury and pneumonia
25	2 10/12	M	55	0	1	Severe inhalation injury
26	30	M	54.5	5.5	36	Acute myocardial infarction
27	28	M	53	39	33	Acute myocardial infarction
28	3	F	53	3	11	*53% total body surface burn with acute suppurative thrombophlebitis and septicemia

* Autopsy not performed

Table 7. Cause of Death, 1981 - Continued

Patient	Age	Sex	% Burn Total	% Burn 3°	PEB	Cause of Death
29	21	F	52	52	17	52% total body surface burn, pneumonia and septicemia
30	2 8/12	M	51.5	38.5	14	*51.5% total body surface burn plus inhalation injury
31	31	M	51.5	15	2	*51.5% total body surface burn plus inhalation injury
32	3	M	50.5	47.5	21	*50.5% total body surface burn and invasive burn wound sepsis
33	69	M	50	36.5	40	*50% total body surface burn, pneumonia and septicemia
34	41	M	48	0	34	48% total body surface burn, severe inhalation injury and pneumonia
35	54	M	45	3	10	45% total body surface burn plus acute myocardial infarction
36	62	M	44	44	1	44% total body surface burn and acute respiratory insufficiency
37	46	M	42	40	31	42% total body surface burn, severe inhalation injury and pneumonia
38	65	M	38	0	38	38% total body surface burn, acute myocardial infarction and pulmonary embolus
39	87	M	33.5	33.5	23	*33.5% total body surface burn, severe inhalation injury and pneumonia
40	50	M	31	31	63	31% total body surface burn, pneumonia and septicemia
41	57	M	26.5	12	19	*26.5% total body surface burn, pneumonia and acute renal failure
42	1 4/12	M	26	10	5	*26% Total body surface burn, pneumonia and septicemia
43	40	M	24.5	19.5	7	*24.5% total body surface burn and severe inhalation injury

* Autopsy not performed

PRESENTATIONS:

Pruitt BA Jr: Current Concepts of Burn Care. Coco Solo Hospital, Panama Canal Zone 12 Jan 81.

Pruitt BA Jr: Recent Advances in Burn Care. Medical Assn of the Isthmian Canal Zone, Panama 12 Jan 81.

Pruitt BA Jr: 1)Pulmonary Complications of Thermal Injury, Including Inhalation Injury; 2) Management of the Burn Wound. Gorgas Army Hospital, Panama 13 Jan 81.

Pruitt BA Jr: Early Care of the Extensively Burned Patient. Santo Thomas Hospital, Panama 14 Jan 81.

Pruitt BA Jr: Management of Burn Patients in a Combat Environment. Gorgas Army Hospital, Panama 14 Jan 81.

Pruitt BA Jr: Metabolic Changes and Nutrition of Burn Patients. Gorgas Army Hospital, Panama 15 Jan 81.

Mansour EH: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 14 Jan 81.

Pruitt BA Jr: Burn Care: From Hopelessness to Hope. Evanston Hospital Burn Center, Evanston, IL 19 Jan 81.

Pruitt BA Jr: Triage and Initial Care of Burns. Robert B. Green Hospital, San Antonio, TX 4 Feb 81.

McManus WF: Classification of Burns. Intensive Care Nurse Clinician Course students, BAMC Ft Sam Houston, TX 4 Feb 81.

McManus WF: Complications of Burns. Intensive Care Nurse Clinician Course students, BAMC Ft Sam Houston, TX 5 Feb 81.

Strieper GE: Burn Nursing. Intensive Care Nurse Clinician Course students, BAMC, Ft Sam Houston, TX 6 Feb 81.

Maguire M: Physical Therapy in Burns. Intensive Care Nurse Clinician Course students, Ft Sam Houston, TX 9 Feb 81.

Fullerton J: Role of Occupational Therapy in the Thermally Injured Patient. Intensive Care Nurse Clinician students, Ft Sam Houston, TX 9 Feb 81.

Pruitt BA Jr: 1)Care of Burn Patients and Combat Environment Modification Thereof; 2) Wound Care and the Diagnosis and Treatment of Inhalation Injury. Brooks Aerospace, San Antonio, TX 11 Feb 81.

Cheney VR: Burn Nursing. Nursing students Brackenridge Hospital School of Nursing, Austin, TX 11 Feb 81.

Terry J: Emergency Care in Burns. Physician's Assistants students AHS, Ft Sam Houston, TX 13 Feb 81.

Cheney VR: Overview of Burn Care. Association of Critical Care Nurses, San Antonio, TX 17 Feb 81.

McManus WF: Modern Trends in Burn Management. Southwest Missouri Chapter American College of Surgeons. San Antonio, TX 20 Feb 81.

Cheney VR: Burn Nursing. Nursing students, Baptist Hospital School of Nursing, San Antonio, TX 23 Feb 81.

Cheney VR: Burn Management. Medical Explorers (Boy Scouts) San Antonio, TX 25 Feb 81.

Pruitt BA Jr: 1) Early Care of the Burn Patient - Minor and Major; and 2) Life-Threatening Complications of Thermal Injury. Wesley Medical Center Trauma - Initial Care Symposium, Wichita, KS 27 Feb 81.

Maguire M: Physical Therapy and Thermal Injuries. Students 91J school, BAMC Ft Sam Houston, TX 4 Mar 81.

Maguire M. Evaluation of the Upper Extremity in Sports. Medical Explorers (Scouts) San Antonio, TX 5 Mar 81.

Pruitt BA Jr: 1) Early Care of the Severely Burned Patient; 2) Metabolic Alterations Following Multisystemic Injury and Implications of Nutritional Management; 3) Nutritional Management of the Severely Injured Patient; 4) Massive Body Burns; 5) Pulmonary Complications Following Massive Body Burn Injuries. USC Critical Care Medicine Course, Las Vegas, NV 5-7 Mar 81

Pruitt BA Jr: Initial Assessment of Burn Patients. Brown University, School of Medicine, Department of Surgery, Providence, 12-14 Mar 81.

McManus WF: Management of the Burn Patient. Army Science Board briefing, Fort Sam Houston, TX 17 Mar 81.

Maguire M: P.T. and the Thermally Injured Patient. USAF P.T. students, Wilford Hall USAF Medical Center, Lackland AFB, TX 17 Mar 81.

Fullerton J: O.T.s Role with Thermally Injured Patients. Social Work Service, Relatives of Patients. Ft Sam Houston, TX 18 Mar 81.

Pruitt BA Jr: Care of Burn Patients in a Combat Environment. US Army Reserve Medical Symposium, Oklahoma City, OK 19 Mar 81.

Maguire M: Care of the Burn Patient. Baylor Univ Master's P.T. students, Academy of Health Sciences, Ft Sam Houston, TX 24 - 25 Mar 81.

The following presentations were made to the Oklahoma Surgical Society, Fort Sam Houston, TX on 26 Mar 81:

Pruitt BA Jr: Current Techniques of Burn Care
McManus WF: Recent Advances in Burn Care.

Mansour EH: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 27 Mar 81.

Pruitt BA Jr: Initial Assessment of Burn Patients. Fort Sam Houston Advanced Trauma Life Support. 29 Mar 81.

Cheney VR: Overview of Burns. Nursing educators, BAMC Recruiting Command, Ft Sam Houston, TX 31 Mar 81.

Pruitt BA Jr: Stress Ulcers and Postinjury Pancreatitis. ACS Spring Meeting, New Orleans, LA 1 Apr 81.

Pruitt BA Jr: Planning, Implementing and Evaluating the Importance of Public Education Programs. American Burn Assn Anl Mtg, Washington, D.C. 3 Apr 81.

Maguire M: The Evaluation of the Lower Leg and Overuse Syndromes. HSC Musculoskeletal Course, Ft Sam Houston, TX 8 Apr 81.

Maguire M: Evaluation and Treatment of the Elbow, Wrist and Hand. HSC Musculoskeletal Course, Ft Sam Houston, TX 14 Apr 81.

Maguire M: Evaluation of the Hip and Its Treatment. HSC Musculoskeletal Course, Ft Sam Houston, TX 15 Apr 81.

Pruitt BA Jr: Initial Care of the Burn Patient. Wilford Hall USAF Medical Center, Lackland AFB, TX 16-18 Apr 81.

Fullerton J: The Role of the Occupational Therapist in the Care of the Burn Patient. Occupational Therapy students, St. Phillip's College, San Antonio, TX 20 Apr 81.

Pruitt BA Jr: 1) Early Care of the Burn Patient; 2) Diagnosis and Treatment of Inhalation Injury: Triage and Aeromedical Transfer of Burn Patients. Brooks AFB Battlefield Medicine Course, San Antonio, TX 29 Apr 81.

Pruitt BA Jr: Review of Clinical and Research Activities of USAISR. HSC for Washington Corps of Military Attaches, Fort Sam Houston, TX 29 Apr 81.

Syby C: Burn Care. Incarnate Word School of Nursing, San Antonio, TX 30 Apr 81.

Pruitt BA Jr: The ISR Experience with Patients Sustaining Burns in Vietnam. Gary Wratten Symposium, San Antonio, TX 1 May 81.

Pruitt BA Jr: Current Military Research in Burn Care. 121st ARCOM Medical Seminar, Birmingham, AL 2 May 81.

Pruitt BA Jr: Gastrointestinal Complications of Injury. El Paso Surgical Society, El Paso, TX 4 May 81.

Pruitt BA Jr: The Metabolic Response to Injury. Texas Tech Regional Academic Health Center at El Paso, El Paso, TX 4 May 81.

Cheney VR: Burn Care. LVN School, Jourdanton, TX 12 May 81.

Pruitt BA Jr: 1) Inhalation Injury to Include Carbon Monoxide Poisoning; 2) The Metabolic Response to Severe Injury; 3) Fluid Replacement Following Injury; 4) The Diagnosis and Treatment of Opportunistic Infections. Barnes Hospital, St. Louis, MO. 13 May 81.

Strieper GE: Burn Care in Disasters. Disaster Planning Workshop, University of Utah, Salt Lake City, UT 14-15 May 81.

McManus WF: Burns. Nursing Inservice Program. Fort Sam Houston, TX 20 May 81.

Cheney VR: Burn Update. Social Work Service, BAMC, Ft Sam Houston, TX 27 May 81.

McManus WF: The Mission and Function of the Institute of Surgical Research. Rotary Club, San Antonio, TX 29 May 81.

McManus WF: Burn Mass Casualty Management. Presented to Second World Congress on Emergency and Disaster Medicine, Pittsburgh, PA 2 Jun 81.

Pruitt BA Jr: The Diagnosis and Treatment of Burn Wound Infections. Robert Packer Hospital, Sayre, PA 3-5 Jun 81.

Cheney VR: Burn Nursing. Brackenridge School of Nursing, Austin, TX 8 Jun 81.

Cheney VR: Overview of Burn Care. Recruiting Command, BAMC Ft Sam Houston, TX 9 Jun 81.

Brown JR: Occupational Therapists Role With Thermally Injured Patients. Social Work Service, patient's relatives. Ft Sam Houston, TX 10 Jun 81.

Cheney VR: Burns as an Emergency. Aviators Academy of Health Sciences, Ft Sam Houston, TX 12 Jun 81.

Pruitt BA Jr: 1) Transportation of Burn Patients; 2) Resuscitation of Burns. Trauma Symposium, Cleveland, OH 12-13 Jun 81.

Cheney VR: Burn Nursing. University of Texas School of Nursing, San Antonio, TX 17 Jun 81.

Pruitt BA Jr: 1) Diagnosis and Treatment of Inhalation Injury; 2) The Metabolic Response and Nutritional Support of the Burn Patient. University of Minnesota Twin Cities, Minneapolis, MN 18-20 Jun 81.

Pruitt BA Jr: Fluid Resuscitation and Metabolic Changes in Burn Patients. Literature Conference UTMC, San Antonio, TX 24 Jun 81.

McManus WF: Electric Injury. Nursing Inservice Program. Fort Sam Houston, TX 24 Jun 81.

Allie J: Physical Therapy and the Burn Patient. 91J students Academy of Health Sciences, Ft Sam Houston, TX 26 Jun 81.

Cheney VR: Overview of Burns. Social Work Service, BAMC, Ft Sam Houston, TX 22 Jul 81.

Brown JR: The Role of Occupational Therapists with the Thermally Injured. 91L students Academy of Health Sciences, Ft Sam Houston, TX 22 Jul 81.

Maguire M: Evaluation of Hip and Upper Extremity in Sports. University of Texas P.T. students, San Antonio, TX 24 Jul 81.

Pruitt BA Jr: Overview of Current Research and Development in the Treatment of Burn Injury. Joint United Kingdom/USN Workshop on Research and Development for Improved Combat Casualty Care, Alverstoke, Hampshire, England 27-31 Jul 81.

Maguire M: Evaluation of Hip and Upper Extremity in Sports. University of Texas P.T. students, San Antonio, TX 31 Jul 81.

McManus WF: The Mission and Function of the Institute of Surgical Research. Rotary Club, San Antonio, TX 10 Aug 81.

Pruitt BA Jr: Burn Care in the Emergency Room. Third Annual USAF PA Seminar, San Antonio, TX 11 Aug 81.

Cheney VR: Burn Care. O.R. Nursing Course, BAMC, Ft Sam Houston, TX 11 Aug 81.

Lawyer RA: Skin Grafting. O.R. Nursing Course, BAMC, Ft Sam Houston, TX 11 Aug 81.

McManus WF: Classification of Burns. Intensive Care Nurse Clinician Course students, BAMC Ft Sam Houston, TX 12 Aug 81.

Pruitt BA Jr: Current Status of Biologic Dressing. Literature Conference UTMC, San Antonio, TX 12 Aug 81.

McManus WF: Complications of Burns. Intensive Care Nurse Clinician Course students, BAMC, Ft Sam Houston, TX 13 Aug 81.

Strieper GE: Care of the Thermally Injured. Intensive Care Nurse Clinician Course students, BAMC, Ft Sam Houston, TX 14 Aug 81.

Allie J: Physical Therapy and the Burn Patient. Intensive Care Nurse Clinician Course students, BAMC, Ft Sam Houston, TX 18 Aug 81.

Brown JR: Occupational Therapy and the Burn Patient. Intensive Care Nurse Clinician Course students, BAMC, Ft Sam Houston, TX 18 Aug 81.

Pruitt BA Jr: Hemodynamic Consequences of Burn Injury. Royal Brisbane Hospital Surgery Grand Rounds, Brisbane, Australia 17 Aug 81.

Pruitt BA Jr: 1)Metabolic Changes After Severe Injury; 2) Current Status of Burn Care. Royal Australasian College of Surgeons, Dunedin, New Zealand 18-21 Aug 81.

The following presentations were made at the course entitled O.T. and P.T. Care in the Thermally Injured Patient. Academy of Health Sciences, Ft Sam Houston, TX 31 Aug - 4 Sep 81:

Maguire M: Physical Therapy and the Burn Patient
Brown JR: Occupational Therapy and the Burn Patient

Pruitt BA Jr: Pulmonary Complications of Thermal Injury. Univ Texas Continuing Medical Education Program, San Antonio, TX 1 Sep 81.

Cheney VR: Overview of Burn Care. Officers Workshop, Academy of Health Sciences, Ft Sam Houston, TX 1 Sep 81.

Allie J: Physical Therapy and the Burn Patient. Social Work Service, Patient's Relatives, Ft Sam Houston, TX 8 Sep 81.

McManus WF: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 15 Sep 81.

Cheney VR: Burn Care. Social Work Service, BAMC, Ft Sam Houston, TX 16 Sep 81.

Allie J: Physical Therapy and the Burn Patient. USAF P.T.s Wilford Hall Medical Center, Lackland AFB, TX 16 Sep 81.

Strieper GE: Pathophysiology of Burns and Burn Care. Graduate Nursing students, University of Texas at San Antonio, TX 18 Sep 81.

Fullerton J: Occupational Therapy and the Burn Patient. Health Careers Class, Highlands High School, San Antonio, TX 22 Sep 81.

Pruitt BA Jr: 1) Early Care of Burn Patients; 2) Burn Wound Care and Complications of Thermal Injury. Battlefield Medicine Course, Brooks AFB, TX 23 Sep 81.

Pruitt BA Jr: Current Status of Burn Care in the United States. San Antonio Shriners Meeting, San Antonio, TX 23 Sep 81.

Strieper GE: Burn Care as Part of Operational Readiness. Navy nurses, National Naval Medical Center, Bethesda, MD 24 Sep 81.

Pruitt BA Jr: Current Status of Biologic Dressings. General Motors Surgical Research Conference, Sloan-Kettering, New York, NY 28 Sep 81.

Cheney VR: Burn Care. Nursing students, University of Texas at San Antonio, TX 29 Sep 81.

Stallings RJ: The Burn Patient. Social Service Brooke Army Medical Center Fort Sam Houston, TX 30 Sep 81.

McManus WF: Inhalation Injury. Nursing Inservice Program. Fort Sam Houston, TX 1 Oct 81.

Cheney VR: Burn Nursing. Physician's Assistants, Academy of Health Sciences, Ft Sam Houston, TX 2 Oct 81.

Cheney VR: Burn Nursing. LVN Assistants, San Antonio, TX 6 Oct 81.

Pruitt BA Jr: Fluid Management of the Extensively Burned Patient. General Surgery Service, BAMC, Ft Sam Houston, TX 6 Oct 81.

Brown JR: O.T.'s Role with the Thermally Injured. 91L students, Academy of Health Sciences, Ft Sam Houston, TX 13 Oct 81.

Strieper GE: Burn Treatment in the NBC Environment. 21st General Hospital, St Louis, MO. 18 Oct 81.

McManus WF: Treatment of Burn Patients. American Medical Record Association, San Antonio, TX 21 Oct 81.

Cheney VR: Burns as an Emergency. Aviators, Academy of Health Sciences, Ft Sam Houston, TX 26 Oct 81.

Pruitt, BA Jr: Current Management of Burn Injury. Wilford Hall Surgical Staff Lecture Series. Lackland AFB, TX 27 Oct 81.

Strieper GE: Overview of Burn Nursing. Nursing students of the University of New Mexico, Albuquerque, NM 27-29 Oct 81.

The following presentations were made at the annual meeting of the Association of Military Surgeons of the US, San Antonio, TX 2 Nov 81:

Pruitt BA Jr: Epidemiology Triage and Transport of the Burn Patient
McManus WF: Resuscitation and Early Care of the Burn Patient.
Goodwin CW: Diagnosis and Treatment of Inhalation Injury
Stallings RJ: Diagnosis, Treatment and Prevention of Burn Wound Infections
Shirani KZ: Burn Wound Closure Including Excision

Cheney VR: Burn Nursing in Disaster. Presented to the Air Force Nurses, Wilford Hall USAF Medical Center, San Antonio, TX 2-3-4 Nov 81.

Yurt RW: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 3 Nov 81.

Allie J: Physical Therapy and the Burn Patient. Social Work Service, patients families, Ft Sam Houston, TX 4 Nov 81.

Pruitt BA Jr: Outpatient Management of Minor Burns. Presenting Emergencies Series, Georgia Hospital Association 4 Nov 81.

Pruitt BA Jr: Metabolic and Nutritional Consequences of Thermal Injury. Bristol Myers Symposium on Nutritional Research. Washington, DC 9 Nov 81.

McManus WF: Traumatic Injury. Clinical Pastoral Chaplain's Course, BAMC, Fort Sam Houston, TX 10 Nov 81.

Brown JR: Occupational Therapy and the Burn Patient. Social Work Service, Patient's Relatives, Ft Sam Houston, TX 11 Nov 81.

Cheney VR: Overview of Burns. Department of Human Resources, San Antonio, TX 20 Nov 81.

Pruitt BA Jr: Modern Burn Therapy. New Jersey Medical School, Newark, NJ 22-24 Nov 81.

McManus WF: Care of the Wounds. Nursing Inservice Program. Fort Sam Houston, TX 25 Nov 81.

Strieper GE: Management of Burn Patients. Missouri State University, Kirksville, MO. For the Recruiting Command. 30 Nov 81.

Strieper GE: Management of Burn Patients. Avila College, Kansas City, MO. For the Recruiting Command. 1 Dec 81.

Strieper GE: Management of Burn Patients. Washburn College, Topeka, KS. For the Recruiting Command. 2 Dec 81.

Strieper GE: Management of Burn Patients. Department of Nursing, Fort Riley, KS. For the Recruiting Command. 3 Dec 81.

Strieper GE: Management of Burn Patients. Pittsburg State College, Pittsburg, KS. For the Recruiting Command. 4 Dec 81.

Pruitt BA Jr: 1) Fluid Resuscitation and Metabolic Changes in the Injured Man; 2) Current Techniques of Burn Care and Treatment of Infections. Philippine College of Surgeons, Manila 7-11 Dec 81.

McManus WF: Lasers and Radiation Burns. Academy of Health Sciences, Fort Sam Houston, TX 8 Dec 81.

McManus WF: Treatment of Burns. Battlefield Medicine course, Brooks AFB, TX 9 Dec 81.

Pruitt BA Jr: 1) Metabolic Consequences of Thermal Injury; 2) Hemodynamic Monitoring of Burn Patients; 3) Unsolved Problems and Needs in Burn Care. International Society for Burn Injury Annual Meeting, Denver, CO 12 Dec 81.

PUBLICATIONS

Aulick LH, Goodwin CW, Becker RA et al: Visceral blood flow following thermal injury. Ann Surg 193:112-116, Jan 81.

Pruitt BA Jr: Fluid resuscitation for extensively burned patients. J Trauma 21, No 8 Suppl: 690-692, Aug 81.

McManus WF, Goodwin CW, Mason AD Jr et al: Burn wound infection. J Trauma 21:753-756, Sep 81.

Aulick LH, Baze WB, Johnson AA et al: A large animal model of burn hypermetabolism. J Surg Res 31:281-287, Oct 81.

Powanda MC and Moyer EB: Plasma proteins and wound healing. SG&O 153:749-755, Nov 81.

Walker HL, McLeod CG and McManus WF: Experimental inhalation injury in the goat. J Trauma 21:962-964, Nov 81.

Goodwin CW, Lam V, Mason AD Jr et al: Colloid and crystalloid resuscitation have same effect on lung water after thermal injury. Surg Forum XXXII: 294-297, 1981.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

REPORT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS--ANESTHESIOLOGY

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 January 1981 - 31 December 1981

Investigator:

Anton J. Jirka, MD, MPH, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

REPORT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS--ANESTHESIOLOGY

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 January 1981 - 31 December 1981

Investigator: Anton J. Jirka, MD, MPH, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

In the period covered in this report, 404 anesthetics were administered to 127 patients, an average of 3.18 anesthetics per patient. The most commonly used anesthetic agent was Enflurane (62.38%), followed by ketamine (25.74%), and nitrous oxide (4.94%). Due to the nature and combinations of procedures now performed, regional anesthesia is seldom used. An automatic oscillometric blood pressure monitor is presently used on all patients.

Anesthesia.

ANESTHESIOLOGY

PREOPERATIVE EVALUATION

Most burn patients are several days postinjury when first seen by the anesthesiologist. In the immediate postburn period, the time is used to gain abundant physiologic data from routine monitoring of various indices: hematologic (hematocrit, electrolytes, liver and renal function tests), pulmonary (arterial blood gases, respiratory rate, daily chest roentgenograms), cardiovascular (blood pressure, central venous pressure, cardiac output measured by use of Swan-Ganz catheters), and renal (urine output, urine chemistry), in addition to the usual preoperative patient interview and physical examination.

All patients, regardless of age, who have electrical injuries have a preoperative electrocardiogram performed to rule out possible myocardial damage.

PREOPERATIVE PREPARATION

All patients are kept NPO after 2400 the day prior to surgery with the exception of children, who may receive clear liquids up to five hours prior to surgery.

Due to extraordinary fluid requirements in most burned patients, an intravenous infusion, if not already in place, is begun the evening prior to surgery.

PREMEDICATION

Glycopyrrolate (Robinul^{R}) 0.005 mg/kg to a maximun dose of .4 mg, is given intramuscularly as premedication 30 minutes prior to anesthesia. Narcotic premedication is no longer routinely used.

FLUIDS

All fluids except hyperalimentation solutions are changed to D_5RL or RL on arrival in the operating room. Hyperalimentation solutions are continued throughout operative procedures.

TYPES OF ANESTHESIA

The pattern of anesthetic administration has changed from previous years and involves a greater use of enflurane and

ketamine and a lesser use of halothane and regional anesthesia. The reasons for this change will be discussed under individual agent headings.) (Table 1)

TABLE 1. PRIMARY AGENTS

AGENT	1979		1980		1981	
	NUMBER	%	NUMBER	%	NUMBER	%
ENFLURANE	324	58.59	252	47.46	252	62.38
KETAMINE	143	25.86	183	34.46	104	25.74
HALOTHANE	18	3.25	10	1.88	16	3.96
N_2O	38	6.87	71	13.37	20	4.95
LOCAL	29	5.24	15	2.82	10	2.48
OTHER	1	0.18	0	0	2	0.49

1. Enflurane (Ethrane^R)

Enflurane is a halogenated ether which has been commercially available for approximately the past seven years. It has a rapid induction with good muscle relaxation. Biotransformation amounts to less than 2% of an inhaled dose, which perhaps accounts for the few clinical toxic effects observed. Plasma fluoride levels in hypermetabolic burn patients during and after Enflurane administration have been measured and found not to be in the toxic range. Enflurane is presently the most commonly used anesthetic agent at the USAISR.

2. Halothane^R (Fluothane)

The use of halothane is avoided mostly for less than rational reasons related to descriptions of probable hepatotoxicity (incidence 0.7 per 1000) in the literature. Previous studies at the Institute of Surgical Research show its repeated use to be safe in the thermally injured patient, and the National Halothane Study showed halothane to be the anesthetic with the best overall mortality rate. It is a smooth anesthetic, unsurpassed as an agent for pediatric patients. This anesthetic is mainly used now for asthmatics, patients with digitalis toxicity, and children. Its use has decreased as we favor ketamine in the young age group.

3. Nitrous Oxide

This agent is used in concentrations of 50% or 60% with oxygen. It is used mainly in conjunction with other analgesic or anesthetic agents. Succinylcholine has not been used for any purpose in this unit for more than six years.

4. Ketamine

This agent is used both IM and IV to produce its characteristic dissociative state, with preservation of basal functions and laryngeal reflexes plus stimulation of the cardiovascular system.

Unfortunately, ketamine shares with its parent compound, phencyclidine, the production of a high incidence of unpleasant hallucinogenic side effects. There seems to have been a "batch" difference in ketamine, and that possessed by ISR in the past had an almost 100% incidence of these effects. New methods of administering the drug, as well as various methods of premedication and patient preparation, appear to have reduced the unpleasant emergence reactions to a level where they are of little consideration in the well selected patient. Laryngospasm, airway obstruction and regurgitation can occur with ketamine. Pronounced blepharospasm prevents its use in eye cases. All ketamine anesthetics, other than in children, are preceded by IV diazepam (0.15-0.2 mg/kg).

5. Subanesthetic Ketamine

Subanesthetic ketamine (single dose 1.5-2 mg/kg IM) has not been used during this reporting period except for dressing changes where it is the anesthetic of choice. Tolerance to ketamine has been noted in several patients after repeated (greater than five) ketamine anesthetics. Ketamine is no longer used for Hubbard tank procedures. Although of limited value, sedation and narcotic analgesia, administered under direction of the surgical staff, have replaced ketamine for this use.

6. Regional Anesthesia

Regional anesthesia is generally considered one of the safest methods available, but its use in the thermally injured patient is limited for several reasons: sepsis and infection of the skin over or near the site of injection are contraindications for use, and multiple-site operations also limit the practicality of this method. Axillary block is the most common regional technique used at USAISR. However the tendency toward multiple procedures has decreased the usefulness of this technique.

MONITORING TECHNIQUES

A. CIRCULATION

1. Precordial and/or esophageal stethoscope

2. Peripheral pulse

3. Blood pressure. Direct arterial lines have been used when necessary. The Dinamap^{R} blood pressure instrument is routinely used for intraoperative blood pressure monitoring. Since it can be used over dressings and is noninvasive, it is a most practical method of monitoring blood pressure in our patient population.

4. CVP

5. Swan Ganz catheter

6. ECG

7. Sponge weight - rarely used

8. Urine output

B. RESPIRATION

1. Rate

2. Auscultation

3. Arterial blood gases

C. TEMPERATURE

In most cases a temperature monitor is employed. Because of the greatly increased evaporative heat losses in burn patients, hypothermia is a serious problem. Several methods are employed to maintain body temperature during anesthesia:

1. Ambient temperature is maintained at 82-87°F. This is probably the most important method to reduce heat loss.

2. The anesthetic gases may be heated and humidified.

3. A circle system which allows partial rebreathing of warm expired gases may be used to minimize heat loss. A Bain Circuite which achieves the same purpose is used in children.

4. Radiant heat lamps

5. The K-thermia heating blanket can also be used. It is probably used most effectively on children weighing less than 10 kg and for cooling febrile patients.

COMPLICATIONS

A 40 year old black male with multiple system complications who had suffered two prior cardiac arrests during his hospitalization sustained a third cardiac arrest post tracheostomy. He was successfully resuscitated but died two days later of complications from his burn. An autopsy was not performed.

PATIENT DATA AND OPERATIVE PROCEDURES

The following two tables illustrate overall anesthetic patient data for the years 1970 through 1981 (Table 2) and recent trends in operative procedures (Table 3).

TABLE 2. OVERALL PATIENT DATA, USAISR (1970-1981)

Year	No. of Patients	No. Patients Anesthetized (ISR Only)	Average Number Patients Anesthetized	Total Anesthetics Given at ISR	Average Anesthetics Per Patients Anesthetized
1970	321	198	61.7	497	2.51
1971	301	179	59.5	475	2.65
1972	301	183	60.8	575	3.14
1973	273	141	51.6	377	2.67
1974	226	123	54.4	380	3.09
1975	254	142	55.9	490	3.45
1976	277	139	50.2	476	3.43
1977	242	129	53.3	344	2.67
1978	268	151	56.3	435	2.88
1979	267	161	60.3	554	3.44
1980	243	148	60.91	531	3.59
1981	208	127	61.06	404	3.18

TABLE 3. NATURE OF SURGERY, USAISR

	1978		1979		1980		1981	
PROCEDURE	NUMBER PROCEDURES	%	NUMBER PROCEDURES	%	NUMBER PROCEDURES	%	NUMBER PROCEDURES	%
EXCISION	90	19.3	212	30.15	269	37.36	212	36.68
AUTOGRAFT	269	59.9	372	52.91	318	44.17	293	50.69
ORTHOPEDIC	33	7.1	34	4.84	38	5.28	23	3.98
CHONDRECTOMY	4	0.9	1	0.14	4	0.56	3	0.52
EYE AND LID	6	1.3	21	2.99	17	2.36	3	0.52
INTRA-ABDOMINAL	6	1.3	8	1.13	1	0.14	1	0.17
PLASTIC	6	1.3	3	0.43	5	0.69	3	0.52
OTHER	50	10.8	52	7.39	68	9.44	40	6.92
TOTAL	464	100%	703	100%	720	100%	578	100%

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OG 6975	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. CHANGE	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AF	161
b. CONTRIBUTING				
c. ~~XXXXXXXX~~	STOG 80 - 7.2:5			

11. TITLE (Procede with Security Classification Code)

(U) The Cardiopulmonary Response to Thermal Injury in Burned Soldiers

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 10	Cont	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	2.0	100
	CURRENT 83	2.0	120

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

ADDRESS: Fort Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL

NAME: Basil A. Pruitt, Jr., MD, COL, MC

TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

NAME: US Army Institute of Surgical Research
Surgical Study Branch

ADDRESS: Fort Sam Houston, Texas 78234

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: Cleon W. Goodwin, Jr., M.D.

TELEPHONE: 512-221-2968

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME:

NAME:

POC: DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Resuscitation Fluids; (U) Burn Injury; (U) Cardiac Output; (U) Cardiovascular Hemodynamics; (U) Indicator Dilution; (U) Humans; (U) Animal Model

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) To evaluate systemic and cardiopulmonary changes in burned soldiers and the influence of fluid resuscitation. To study by both invasive and noninvasive techniques pulmonary and myocardial function in burned and burned-infected patients.

24. (U) Hemodynamic flow and pressure changes are studied in burn patients during and after resuscitation. Cardiac output and lung water are studied by a standardized rebreathing indicator-dilution technique and by echocardiography. Comparisons in cardiac output between these two methods are made and the state of myocardial contractility is determined.

25. (U) 8110 - 8209. To assess the effects of crystalloid and colloid resuscitation on hemodynamic response and on lung water following thermal injury, 75 patients (mean age 28 years, range 18-44, mean burn size 47% total body surface, range 20-80%) were randomly assigned to receive lactated Ringer's solution (CRYS) or a 2.5% albumin-lactated Ringer's solution (COLL) administered at a rate to produce a urinary output of 30-50 ml/hr. Cardiac output and myocardial contractility were determined by echocardiography, and pulmonary extravascular water was measured by a standard rebreathing technique. COLL patients received less fluid than did CRYS to restore adequate organ function ($p < .01$). Restoration of cardiac output was identical, and indices of myocardial

*Available to contractors upon originator's approval

performance were supranormal ($p < .01$ vs control) and equal in the two resuscitation groups. Lung water remained unchanged in CRYS patients and progressively increased in the COLL patient ($p < .001$) over the 7 day study; however, mean lung water was not significantly different between the two groups. The inclusion of colloid in the resuscitation fluid did not reduce measured lung water and may have produced the opposite effect. The depressed cardiac output following thermal injury is due to decreased intravascular volume rather than to a myocardial depressant factor.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

REPORT TITLE: THE CARDIOPULMONARY RESPONSE TO THERMAL INJURY IN BURNED SOLDIERS - RANDOMIZED TRIAL OF EFFICACY OF CRYSTALLOID AND COLLOID RUSUSCITATION ON HEMODYNAMIC RESPONSE AND LUNG WATER FOLLOWING THERMAL INJURY

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1981 - 30 September 1982

Investigators:

Cleon W. Goodwin, M.D.
James Dorethy, M.D.
Victor Lam, M.D.
Basil A. Pruitt, Jr., M.D.

Reports Control Symbol MEDDH-283(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

REPORT TITLE: THE CARDIOPULMONARY RESPONSE TO THERMAL INJURY IN BURNED SOLDIERS - RANDOMIZED TRIAL OF EFFICACY OF CRYSTALLOID AND COLLOID RESUSCITATION OF HEMODYNAMIC RESPONSE AND LUNG WATER FOLLOWING THERMAL INJURY

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1981 - 30 September 1982

Investigators: Cleon W. Goodwin, M.D.
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Victor Lam, M.D.
Basil A. Pruitt, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

To assess the effects of crystalloid and colloid resuscitation on hemodynamic response and on lung water following thermal injury, 79 patients were randomly assigned to receive lactated Ringer's solution or 2.5% albumin-lactated Ringer's solution. Crystalloid treated patients required more fluid for successful resuscitation than did those receiving colloid solutions (3.81 vs 2.98 ml/kg body weight/% body surface burn, $p < 0.01$). In study phase 1 (29 patients), cardiac index and myocardial contractility (ejection fraction and mean rate of internal fiber shortening, V_{cf}) were determined by echocardiography during the first 48 hours postburn. Cardiac index was lower in the 12-24 hour postburn interval in the crystalloid group, but this difference between treatment groups had disappeared by 48 hours postburn. Ejection fractions were normal throughout the entire study, while V_{cf} was supranormal ($p < .01$ vs normals) and equal in the two resuscitation groups. In study

Resuscitation
Lung water
Echocardiography
Colloids
Myocardial depressant factor

phase 2 (50 patients), extravascular lung water and cardiac index were measured by a standard rebreathing technique at least daily for the first postburn week. Lung water remained unchanged in the crystalloid treated patients ($p > 0.10$) but progressively increased in the colloid treated patients over the seven day study ($p < 0.0001$). Measured lung water differed significantly ($p < .001$) between the treatment groups. Cardiac index increased progressively and identically in both treatment groups over the study period ($p < 0.01$). These data refute the existence of myocardial depression during postburn resuscitation and document hypercontractile left ventricular performance. The addition of colloid to crystalloid resuscitation fluids produces no long lasting benefit on total body blood flow and promotes accumulation of lung water when edema is being reabsorbed from the burn wound.

THE CARDIOPULMONARY RESPONSE TO THERMAL INJURY IN BURNED SOLDIERS - RANDOMIZED TRIAL OF EFFICACY OF CRYSTALLOID AND COLLOID RESUSCITATION OF HEMODYNAMIC RESPONSE AND LUNG WATER FOLLOWING THERMAL INJURY

Thermal injury of sufficient duration and intensity causes coagulation necrosis and cell death in the affected tissue. Loss of capillary integrity leads to loss of isotonic fluid from the intravascular space into the tissue surrounding the injury, and in burns exceeding 25% of the total body surface, additional plasma volume may be lost into the unburned tissue (1). Massive edema may form in the burned tissue, and its severity depends on both the extent and depth of injury and on the volume of infusate. With the loss of intravascular volume, cardiac output, blood pressure and urinary output fall, and if the intravascular volume deficits are of sufficient magnitude and are not replaced, acidosis and hypovolemic shock ensue. The loss of plasma volume is too rapid and too massive in patients with extensive burns to allow effective restoration of the intravascular volume deficit by the translocation of fluid from the interstitial and intracellular compartments.

With adequate fluid resuscitation, the fall in plasma volume and total body blood flow can be limited. Although cardiac output is usually restored to near normal levels during the latter half of the first 24 hours postburn, plasma volume is not restored to normal levels until the end of the second postburn day (2). While the net plasma volume deficit is dependent upon the amount of infused resuscitation fluid, the rate of plasma volume loss into the surrounding tissue is not affected by fluid restoration during the first 18 to 24 hours following injury. Subsequently, capillary integrity returns to normal, fluid infusion effectively restores intravascular volume, and cardiac output rises to supranormal levels typical of the early postinjury hypermetabolic response (3). The rate of fluid infusion is dictated by the patient's physiologic response to resuscitation. Animal studies of organ blood flow distribution

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indicate that the kidney is the most poorly perfused organ following thermal injury (4). By implication, when renal function is adequate, other vital organs usually are being perfused satisfactorily, and urinary output is the most reliable and readily accessible index of effective resuscitation.

Before the realization that severe thermal injury was associated with massive loss of isotonic fluid into the injured tissues, a syndrome of "burn shock" was described, in which thermally injured patients failed to respond to the then customarily administered quantities of fluid (5). Subsequently, the effectiveness of massive quantities of balanced electrolyte solutions in replacing not only the intravascular volume deficit but also that of the entire functional extracellular space was demonstrated (6), and the use of such volume replacement has virtually eliminated renal failure and cardiovascular collapse as a cause of early postburn death. The failure of cardiac output to return rapidly to normal following infusion of fluid volumes estimated to be necessary for adequate resuscitation has been ascribed to the presence of a circulating myocardial depressant factor (6,7). Myocardial depression also has been postulated to explain the inability of fluid infusions to reestablish organ perfusion in certain categories of burned patients, especially those at either extreme of age (8). However, the existence of such a myocardial depressant factor has been proposed on the basis of decreased cardiac output, and this hemodynamic variable does not directly indicate myocardial performance. Direct measurement of left ventricular myocardial contractility during the immediate postburn period has not been reported.

The lung participates in the pathophysiological alterations associated with large plasma volume losses and administration of large resuscitation volumes following thermal injury. In the

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absence of inhalation injury, successful resuscitation commonly restores systemic and pulmonary hemodynamic indices to normal with no subsequent pulmonary complications. The pulmonary response to thermal injury in humans is less well described. Inhalation injury accentuates fluid requirements during resuscitation and predisposes to the development of acute pulmonary edema during the first postburn week (9). Early pulmonary edema may also occur in patients with no coexisting inhalation injury or preexisting cardiovascular disease when edema in the burn wound is being rapidly mobilized during the fourth to the eighth postburn days.

The formulas commonly used to estimate the resuscitation fluid needs of burned patients vary widely in terms of both the volume and composition of the fluids recommended. The majority of patients show a satisfactory clinical response to resuscitation no matter which formula is used to predict fluid requirements. This observation is a reflection of the physiologic tolerance of the patients treated, since the volume dosage and salt dosage of the various formulas for the first 24 hours postburn alone differ by more than twofold. Although virtually all formulas provide for administration of colloid-containing fluids in the second 24 hours postburn, the recommended colloid-containing fluid for the initial 24 hours postburn ranges from a volume equal to that of electrolyte-containing fluid administered to no colloid-containing fluid at all. As in the case of resuscitation of other trauma patients, controversy exists over whether colloid-containing fluids are necessary, desirable, or even deleterious. Proponents of colloid-containing fluid as a part of initial postburn resuscitation have claimed that inclusion of such solutions reduces the volume of fluid required for resuscitation, maintains urinary output at a higher level than with an equal volume of crystalloid fluid, supports cardiac output, and minimizes loss of fluid into the pulmonary interstitium and other tissues. Conversely, many feel that the immediate postburn increase in capillary permeability permits leakage of blood-borne colloid and that colloid-containing fluid is retained within the circulation to no greater extent than an equal volume of non-colloid electrolyte solution in the immediate postburn period (10) That school also considers that colloid-containing fluid has little, if any, effect on cardiac output above that of an equal volume of electrolyte-containing fluid, has no specific beneficial

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effect in terms of change in lung water volume, and in fact, may be deleterious when given in large amounts (11).

To compare the effect of resuscitation solution composition on myocardial performance and lung water following thermal injury, we studied 79 patients who were randomized to receive crystalloid or colloid-containing resuscitation solutions. Our results indicate that the addition of colloid to crystalloid solutions produces no important hemodynamic benefits and is associated with increased accumulation of lung water after the immediate resuscitation. In neither treatment group was any evidence of myocardial depression documented, and in fact, the myocardium was hypercontractile within 12 hours of injury.

MATERIAL AND METHODS

Patient Sample

Seventy-nine thermally injured patients were serially studied after obtaining informed consent for participation in research protocols approved by institutional review (Table 1). Control of resuscitation was obtained within four hours of injury, and all patients were admitted within twelve hours of injury. Patients were assigned by a random numbers table to receive either crystalloid or colloid resuscitation. Patients in the crystalloid arm were given lactated Ringer's solution and those in the colloid arm were given 2.5 albumin-lactated Ringer's solution. During the first 24 hours, fluid was administered at a rate sufficient to stabilize vital signs and to produce a urinary output of 30 to 50 ml/hr. Resuscitation requirements for each treatment group are indicated in Table 1. Plasma volume was replaced on the second postburn day by colloid equivalent to plasma in a dosage of 0.3 to 0.5 ml/kg body weight/% body surface burn. Following the initial 24 hour resuscitation phase, 5% dextrose in water was administered at a rate which allowed each patient's weight to return to preburn levels by postburn day 7 to 10 and which maintained serum sodium and osmolal concentrations in the normal range. No patients had evidence of inhalation injury or other pulmonary disease based on clinical evaluation and on normal fiberoptic bronchoscopy, ^{133}xenon ventilation-perfusion lung scan, chest roentgenogram,

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and arterial blood gases. None of the patients demonstrated microbiological or clinical evidence of pulmonary infection during the seven days of the studies. The patients were studied in two consecutive phases. Echocardiographic indices of myocardial performance were measured in the first 29 patients, and serial changes in lung water following resuscitation were determined in the next 50 patients.

Echocardiography Protocol

Myocardial performance was determined in three designated resuscitation time periods: initial postburn period (0-12 hours), middle postburn period (12-24 hours), and late postburn period (24-48 hours). M-mode echocardiograms were recorded by an Ekoline 20 Ultrasonoscope (Smith Kline Instruments) and a 2.25-MHz focused transducer (Model C-11A). The analogue signals were recorded by a rapid response ultraviolet photographic recorder (Model 1858, Honeywell Instruments). Patients were examined in the supine position, and reproducible comparisons were insured by the consistent placement of the transducer using intracardiac landmarks and assuring transducer orientation to specific cardiac structures (12). End diastole was defined by the R-wave of the electrocardiogram QRS complex and end systole by the smallest septal-posterior wall endocardial distance. Echocardiograms were digitized on a mini-computer (Model 9830, Hewlett Packard, Inc.), and left ventricular dimensions were then averaged over five beats and used to calculate indices of myocardial performance by standard formulas (13). The measurements of left ventricular size and function by M-mode echocardiography correlate very highly with those of cineangiography (14). Thermodilution cardiac output measurements using iced 5% dextrose solution were calculated from the mean of three consecutive measurements. Normal values for echocardiographic indices and cardiac output were obtained courtesy of the Brooke Army Medical Center Cardiac Catheterization and Noninvasive Laboratories.

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Lung Water Protocol

Lung water and cardiac output were measured every twelve hours (0600 and 1800 hours) for the first three postburn days and once daily (0600 hours) on postburn days five and seven. Extravascular lung water and cardiac output were determined by a standard rebreathing method utilizing two gases of differing solubility. Lung tissue volume measured by this method has been known to reflect with high reliability changes in lung water content in both animals and human subjects with normal and edematous lungs (15-19). After several minutes of quiet breathing to become accustomed to the mouthpiece and noseclip, the patient exhaled to residual volume and began breathing into a reservoir bag containing 1.5% dimethyl ether (soluble gas), 7% helium (insoluble gas), 30% oxygen, and balance nitrogen. Six to eight maximal rebreathing maneuvers were carried out for 15 to 20 seconds. The concentrations of each test gas were measured by a time of flight medical mass spectrometer (MGA 1100A, Perkin Elmer Corp.). Changes in reservoir bag volume were measured with a previously calibrated data acquisition dry spirometer (Model 843, Ohio Instrument Company). A fiberoptic photographic recorder (Model 1858, Honeywell, Inc.) with a frequency response of 5000 Hz recorded the electrical output of the helium, dimethyl ether, and bag volume signals. The signal tracings and calibration standards were digitized off-line from the photographic paper by a mini-computer (HP 9830), which corrected the raw data for time of passage of gases through the sampling system, for gas consumption by the mass spectrometer (60 ml/min), and for anatomic and apparatus dead space in the first end expiratory volume cycle.

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The disappearance of the soluble gas was plotted on semilogarithmic paper so that its slope (pulmonary capillary blood flow) and its time zero intercept (tissue volume) could be calculated. To detect tracer gas recirculation, indicated by a decrease in the logarithmic washout slope, serial least squares lines were calculated through at least three of the first six rebreathing points and the time zero intercept. The line yielding the best squared correlation coefficient was chosen for subsequent calculations. All measurements were made in duplicate, and intervals of at least five minutes between each study were observed to allow exhalation of any soluble gas that may have accumulated in the body. In order to compare measurements among individuals of different sizes, lung water is expressed as milliliters per milliliter of alveolar volume for each patient. The normal range in this laboratory is 0.110 to 0.120 ml/ml alveolar volume. In the absence of significant pulmonary shunting, pulmonary capillary blood flow is identical to cardiac output. Thermodilution cardiac outputs were measured in conjunction with the rebreathing measurements in selected patients.

Statistical Analyses

Data describing patient characteristics are reported as mean $\pm$ SD, while experimentally derived data are reported as mean $\pm$ SEM. A one-way analysis of variance was used to examine serial changes of physiologic indices within each treatment group with time. A two-way analysis of variance was used to detect treatment differences between the crystalloid and colloid groups. When physiologic indices of the treatment groups were compared to the reported values for the normal subjects, statistical difference was assessed with a one-tailed test utilizing the t-distribution (20). Statistical differences with $p < 0.05$ were accepted as significant.

RESULTS

Echocardiographic Protocol

Echocardiographic measurements of myocardial performance were carried out in 29 patients who were randomized to two treatment arms: 15 received colloid-containing fluid and 14 received crystalloid-containing fluid. The mean ages and area of total body surface burn were 27 $\pm$ 10 years and 58 $\pm$ 20% for the colloid arm and 29 $\pm$ 12 years and 55 $\pm$ 21% for the patients in the

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crystalloid arm. The colloid treated patients received 3.12 ± 0.93 ml/kg body weight/% body surface burn during the first 24 hours following injury, while the crystalloid treated patients received 3.94 ± 2.24 ml/kg body weight/% burn. In contrast to the combined data for both protocols (Table 1), the difference in resuscitation requirements between these two treatment groups was not statistically significant because of the large variability of the fluid volume administered to the crystalloid group. Urinary output of the colloid treated patients was higher than that of the crystalloid treated patients (65 ± 30 ml/hr vs 51 ± 22 ml/hr); however, that difference between the two treatment groups demonstrates only a trend toward statistical significance ($p=0.08$).

Left ventricular ejection indices were measured in three consecutive time intervals. Ejection fractions were normal throughout the entire study (Fig. 1). Ejection fractions of the colloid group for each defined study interval are: 0.78 ± 0.02, 0.74 ± 0.01, and 0.75 ± 0.02. Corresponding values for the crystalloid group are: 0.79 ± 0.02, 0.75 ± 0.02, and 0.75 ± 0.02. The normal echocardiographic ejection fraction is 0.74 ± 0.02. No statistical differences were evident between treatment groups, across time, or between patient groups and the normal population. The mean rate of internal circumferential fiber shortening (V_{cf}) was in the hypercontractile range in both treatment groups (Fig. 2). In the colloid treated patients, V_{cf} was 1.59 ± 0.16 in the 0-12 hour interval, 1.86 ± 0.11 in the 12-24 hour interval, and 1.64 ± 0.14 in the 24-48 hour interval. V_{cf} in the crystalloid group was 1.72 ± 0.08 in the 0-12 hour interval, 1.68 ± 0.10 in the 12-24 interval and 1.70 ± 0.09 in the 24-48 hour interval. Normal V_{cf} is 1.22 ± 0.06 circumference/second (circ/sec). V_{cf} in each treatment group at all time intervals was statistically similar. However, all values for V_{cf} in both treatment groups are increased above normal ($p < 0.05$).

The serial changes in cardiac indices and left ventricular volume during the first 48 postburn hours are listed in Table 2. Patients in the crystalloid treated group had a significantly lower cardiac index in the 12-24 hour period when compared to the colloid treated patients ($p < 0.01$). This decrease in cardiac index was documented by both echocardiographic determinations and thermodilution techniques. However, by 48 hours postburn, this difference had disappeared, and the cardiac indices in both treatment groups had risen significantly above those determined shortly following admission ($p < 0.05$). End diastolic volume index and stroke index in both treatment groups were below normal values in the first study period ($p < 0.05$), indicating an early intravascular volume deficit. In contrast to colloid treated

patients, whose indices returned to normal, end disastolic volume index and stroke index in the crystalloid treated patients remained significantly depressed in the 12-24 hour study interval ($p < 0.01$). These volume indices were obtained simultaneously with the cardiac index measurements and indicate decreased intravascular volume in this time period. However, by 48 hours, these differences between treatment groups had disappeared. Although end diastolic volume index and stroke index in both treatment groups at this time did not differ significantly from predicted normal values, they were slightly depressed, with no evidence of fluid overload.

Lung Water Protocol

In the second phase of this study, 50 patients were randomized consecutively into two treatment groups of 25 patients each to receive either colloid or crystalloid fluid for resuscitation. The patients' mean age was 29 $\pm$ 8 years in the colloid group and 27 $\pm$ 9 years in the crystalloid group, while their burn sizes were 50 $\pm$ 20% and 43 $\pm$ 12% of the body surface, respectively. Neither characteristic is significantly different between treatment groups. The crystalloid treated patients received significantly more fluid (3.74 $\pm$ 1.28 ml/kg body weight/% burn) than did the colloid treated patients (2.89 $\pm$ 1.27 ml/kg body weight/% burn, $p < 0.01$). By the end of the seven day study, five patients in the colloid treated group demonstrated roentgenographic evidence of pulmonary edema, as did one patient in the crystalloid treated group. Eleven paients receiving colloid resuscitation died later during their hospital courses, while three patients treated with crystalloid resuscitation eventually died.

The serial changes in lung water and cardiac index over the seven day study period are outlined in Table 3 (Fig. 3). Lung water in the colloid treated patients increased significantly during the first postburn week ($p < 0.0001$). In contrast, lung water in the crystalloid treated patients did not change significantly during the seven day study ($p > 0.10$). Measured lung water differed significantly between treatment groups ($p < 0.001$). The effect of resuscitation fluid composition is further demonstrated when lung water is evaluated as a linear function of time postburn by the regression equations LW(COLL) = 0.116 + 0.009 PBD, $r^2 = 0.87$, and LW(CRYS) = 0.128 + 0.003 PBD, $r^2 = 0.43$ (Fig. 4).

Cardiac indices increased significantly during the seven day period of study ($p < 0.01$). At no point during this study were significant differences in cardiac index found between treatment groups.

DISCUSSION

All of the patients reported in these studies were in the young adult age group and none had clinical evidence or a history of heart disease. Coexisting inhalation injury was excluded on the basis of diagnostic criteria having an accuracy of 96% (21). In both of these protocols, resuscitation fluid for the first 24 hours consisted of either lactated Ringer's solution or lactated Ringer's solution containing 2.5% albumin (2.5 gm/dl). Volume requirements were estimated as 2 ml/kg body weight/% burn and the actual infusion rate was adjusted to maintain urinary output at 30 to 50 ml/hr. The colloid treated patients in the overall series required significantly less fluid than did the crystalloid treated patients. This difference did not approach statistical significance in the smaller group of patients evaluated by the echocardiography protocol, in part because the patients receiving colloid-containing solutions were administered fluid at a rate which exceeded the above mentioned guidelines to resuscitation (65 ml/hr for colloid patients and 51 ml/hr for crystalloid patients).

Noninvasive M-mode echocardiographic assessment of cardiac function revealed that cardiac index in the crystalloid group was significantly lower than that of the colloid group, 2.75 $L/min/m^2$ vs 4.6 L/min m^2, in the 12-24 postburn hour interval. Cardiac index in the former group was 81% of predicted normal and was not associated with any clinical evidence of inadequate vital organ function. Cardiac index in the group receiving colloid-containing fluids was 137% of predicted normal, and it is not at all certain that a supranormal cardiac output is of any physiologic benefit during postburn resuscitation. Thermodilution cardiac indices were systematically lower but paralleled those determined by echocardiography in all the periods. Both methods confirm that colloid-containing solutions more rapidly restore depressed cardiac output than do crystalloid-containing solutions. However, by the end of the second postburn day, when plasma deficits have been repleted, cardiac indices have returned to high normal levels in both groups.

Assessment of myocardial contractility in the two groups revealed that ejection fraction was identical in both groups at all time periods and did not vary significantly from predicted

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21. Agee RN, Long JM III, Hunt JL, Petroff TA, et al. Use of ^{133}xenon in early diagnosis of inhalation injury. J Trauma 16:218-224, 1976

normal. The mean rate of left ventricular internal fiber shortening (V_{cf}) showed no depression in either group at any measurement time in the first two postburn days. No decrease in V_{cf} was observed even in the group receiving only crystalloid resuscitation in the 12-24 hour postburn interval, when cardiac index was depressed. In fact, V_{cf} was supranormal at all times in both groups, indicating a hyperdynamic, not a depressed, myocardium. Such a physiologic state might well be anticipated in light of the early postburn outpouring of catecholamines (22).

Measurement of left ventricular end diastolic volume index and stroke index revealed similar depressions below predicted normal values in both groups during the first 12 postburn hours and in the crystalloid group in the 12-24 hour interval. These findings implicate an intravascular volume deficit as the cause of the decreased cardiac index noted in the crystalloid group and, when interpreted in conjunction with the measurements of left ventricular ejection fraction and V_{cf}, provide convincing evidence against the presence of a circulating myocardial depressant factor. During the 24-48 hour postburn interval, these left ventricular volume indices had returned to essentially normal levels in both groups. Colloid resuscitation appears to be associated with earlier intravascular volume restitution compatible with increased intravascular retention of colloid as compared to crystalloid during the latter half of the first postburn day, suggesting a restoration of functional capillary integrity at this time.

In the studies of lung water, the colloid treated and crystalloid treated patients had burns of similar extent, but the former group required significantly less fluid to achieve clinically adequate resuscitation. Hourly urinary output was adequate in both groups. As in the earlier study group, cardiac output was higher in the colloid treatment group at both 12 and 24 hours postburn, but this difference was statistically insignificant. Cardiac output in the two groups was statistically indifferent to resuscitation fluid composition across the entire duration of the study. The discrepancy in cardiac output between the two phases of this overall study may be explained by the larger volume of colloid administered to the patients in the echocardiographic study, which produced the supranormal cardiac outputs observed in those patients during the 12 to 24 hour postburn interval.

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22. Birke G, Duner H, Liljedahl SO, Pernow B, et al. Histamine, catecholamines and adrenocortical steroids in burns. Acta Chir Scand 114:87-98, 1958

Using a noninvasive rebreathing technique, measured lung water was found to be influenced by composition of resuscitation solutions. While lung water content in colloid treated patients increased significantly during the seven day study, lung water in patients receiving only crystalloid fluids remained unchanged during that study interval. The differential effect of treatment on each group was statistically significant. Both groups displayed qualitatively similar responses in lung water following thermal injury. During the initial 36 hours following injury, lung water in both groups tended to decrease. At that point, lung water in the crystalloid treated group returned to levels found immediately after injury. Patients receiving colloid-containing fluids demonstrated a progressive rise in lung water beginning at the end of the second postburn day and continuing until the end of the study, greatly exceeding the original admission values. This phase corresponds clinically to the reabsorption of burn wound edema which occurs following resuscitation.

The validity of the rebreathing method for estimating lung water requires a brief examination. The volume in which the soluble tracer gas distributes during rebreathing measures lung tissue volume, not water volume. However, since water comprises over 80 percent of the lung tissue volume, the tissue volume measurements reflect primarily lung water content (23). Moreover, the solid structures of the lung can reasonably be assumed to remain constant during the time of the study, and any change in lung tissue volume represents a change in lung water content. Since lung size is variable even in patients of the same height and weight, measured lung water was normalized by each patient's simultaneously measured alveolar volume. If anything, this approach may lead to underestimation of lung water, especially in those patients developing clinically significant pulmonary edema, since the tracer gas will not enter nonventilating portions of the edematous lung. We attempted to avoid patients likely to develop pulmonary edema during the first postburn week, but a few patients developed radiologic evidence of interstitial edema. Since this complication occurred primarily in colloid treated patients, their progressive increase in lung water may be underestimated.

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23. Cander L, Forster RE. Determination of pulmonary parenchymal tissue volume and pulmonary capillary blood flow in man. J Appl Physiol 14:541-551, 1959

The goals of fluid resuscitation are the restoration of vital organ function and establishment of hemodynamic stability at the least physiologic cost. In thermal injury, as in most situations of severe nonhemorrhagic fluid depletion, major deficits of the interstitial and intracellular compartments coexist with the more clinically obvious intravascular volume depletion (24). While colloid solutions primarily replace intravascular deficits, crystalloid solutions will rapidly and more completely replenish all compartments. Early studies of burn injury demonstrated that colloid-containing solutions administered to animal models more rapidly restored cardiac output to normal than did crystalloid solutions when administered on an equal volume basis (25). However, both types of solutions produced identical effects on vital signs, pulmonary and systemic vascular resistance, arterial blood gases, plasma lactate, and lung histology. When indices of adequate intravascular fluid volume, such as venous filling pressure or urinary output, serve as guidelines for fluid administration, colloid and crystalloid solutions appear to be equally effective in restoring cardiac output and hemodynamic stability (26,27). To achieve comparable hemodynamic effects, larger volumes of crystalloid solution must be administered, usually between 2 to 4 times the equivalent volume of colloid solutions. As a result, patients resuscitated with crystalloid solutions gain more weight, develop more peripheral edema, and have a lower plasma oncotic pressure than do similar patients resuscitated with colloid-containing solutions.

In an animal model, Moylan found that sodium and fluid volume doses exert independent effects on the early postburn restoration of cardiac output, with one mEq of sodium exerting the same hemodynamic effect as approximately 13 ml of salt-free fluid volume (10). In that study, restoration of cardiac output was little influenced by inclusion of colloid in the resuscitation regimen. In our patients, colloid solutions failed to demonstrate any clinical advantage over crystalloid solutions

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24. Shires T, Colin D, Carrico J, Lightfoot S. Fluid therapy in hemorrhagic shock. Arch Surg 88:688-693, 1964

25. Asch MJ, Feldman RJ, Walker HL, Foley FD, et al. Systemic and pulmonary hemodynamic changes accompanying thermal injury. Ann Surg 178:218-221, 1973

26. Siegel DC, Cochin A, Geocaris T, Moss GS. Effects of saline and colloid resuscitation on renal function. Ann Surg 177:51-57, 1972

27. Virgilio RW, Rice CL, Smith DE, James DR, et al. Crystalloid vs colloid resuscitation: Is one better? Surg 85: 129-139, 1979

when resuscitation was guided by standard clinical indices, such as blood pressure, pulse rate, and hourly urinary output. Pulmonary capillary wedge pressure in our patients was characteristically below five torr during resuscitation and remained below 10 to 12 torr for the remainder of the postburn week. Any attempt to guide fluid infusion rate during resuscitation by elevating pulmonary capillary wedge pressure or cardiac output into the normal range, particularly with crystalloid patients, caused marked increase in urinary output and did not further improve other vital signs. Weight gain and peripheral edema did not indicate overexpansion of the intravascular volume or compromise of organ function.

Thermal injury is associated with significant alterations in pulmonary microvascular dynamics (28,29). In both clinical and laboratory studies, elevation of pulmonary artery pressure and pulmonary vascular resistance have been measured within the first 12 hours postburn (30). Some investigators have related these changes in patients to the effect of fluid resuscitation and have considered pulmonary systolic arterial pressure to correlate with interstitial pulmonary fluid (31). Others have considered the changes to reflect acute lung injury, particularly inhalation injury. In our study patients, neither resuscitation regimen was associated with elevated pulmonary artery pressure above the normal range, suggesting that neither regimen produced pulmonary edema during resuscitation and that screening for inhalation injury in these patients was effective. The measurements of lung water in the first two days postburn confirms in both treatment groups the absence of pulmonary edema.

The relationship between changes in pulmonary artery pressure and vascular resistance and changes in lung water appears to be dependent upon the primary site of flow resistance.

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28. Demling RH, Will JA, Belzer FO. Effect of major thermal injury on the pulmonary microcirculation. Surg 83: 746-751, 1978

29. Harms BVA, Bodai BI, Kramer GC, Demling RH. Microvascular fluid and protein flux in pulmonary and systemic circulations after thermal injury. Microvasc Res 23:77-86, 1982

30. Martyn JAJ, Snider MT, Szyfelbein SK, Burke JF. Right ventricular dysfunction in acute thermal injury. Ann Surg 191: 330-335, 1980

31. German JC, Allyn PA, Bartlett RH. Pulmonary artery pressure monitoring in acute burn management. Arch Surg 106: 788-791, 1973

If the increase is precapillary, as would be consistent with the measurements of cardiac output and pulmonary capillary wedge pressure in our studies and those of others, one would not anticipate an increase in lung water. If the site of the increased resistance is at the capillary or postcapillary level, as would occur with left ventricular failure or direct capillary injury, one would expect an increase in lung water. The rarity of pulmonary edema in burn patients during resuscitation suggests that the increase in pulmonary vascular resistance resides at a precapillary site. The similarity in lung water changes during the first 48 postburn hours in the two treatment groups reflects the similarity of changes of pulmonary hemodynamic indices in both groups and speaks against a specific effect of colloid on transcapillary movement of fluid in the lung following cutaneous thermal injury. Since protein sieving by the pulmonary microvasculature appears to remain normal during postburn resuscitation (29), the infusion of colloid at this time appears to protect intravascular volume and to inhibit fluid loss into the pulmonary interstitium. This hypothesis is supported by the slight fall in measured lung water in both treatment groups during the first 36 hours following burn injury.

The fall of plasma oncotic pressure in burn patients following massive crystalloid resuscitation is not associated with an increase in pulmonary extravascular lung water (32,33). In animal models of other hypovolemic states, infusion of colloid-containing fluid has been associated with a greater increase in lung water than occurred with infusion of crystalloid fluid (34,35). Albumin is widely distributed throughout the

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32. Tranbaugh RF, Lewis FR, Christensen JM, Elings WB. Lung water changes after thermal injury: the effects of crystalloid resuscitation and sepsis. Ann Surg 192:479-488, 1980

33. Lam V, Goodwin CW Jr, Treat RC, Martin DL, Mason AD Jr, Pruitt BA Jr. Does pulmonary extravascular water vary with colloid oncotic pressure after burn injury. Am Rev Respir Dis 119:139, 1979

34. Schloerb PR, Hunt PT, Plummer JA, Cage GK. Pulmonary edema after replacement of blood loss by electrolyte solutions. Surg Gynecol Obstet 135:893-896, 1972

35. Holcroft JW, Trunkey DB. Extravascular lung water following hemorrhagic shock in the baboon: comparison between resuscitation with Ringer's lactate and plasmanate. Ann Surg 180:408-417, 1974

body, with two-thirds located in extravascular sites. Injected albumin is distributed across the capillary membrane according to biphasic kinetics, characterized by a fast exchange rate and by a much slower exchange rate (36,37). Albumin infused during resuscitation will thus equilibrate across the pulmonary capillary, even if protein sieving is unaffected by burn injury. The extracellular albumin present in the lung following resuscitation will promote subsequent fluid retention within the lung interstitium. Since this occurs at the time of rapid mobilization of burn wound edema fluid, the albumin may exert a subtraction effect on the intravascular fluid volume (11). This hypothesis is supported by the significantly greater lung water measured in the colloid treated group at seven days. Clinical observations are consistent with this concept: five of the colloid treated patients showed roentgenographic changes consistent with early pulmonary edema by the seventh postburn day, while only one crystalloid treated patient demonstrated this complication.

We used colloid solutions containing 2.5% albumin, and the average patient in the colloid treatment group received 300 to 350 grams of albumin during the first 24 hours following burn injury. This colloid concentration is similar to the albumin concentration recommended in the Evans formula (38). In studies assessing the effect of varying doses of colloid on resuscitation and survival following hypovolemic shock, 2 gm/kg body weight of albumin produced the optimal beneficial effect (39). Six percent colloid solutions were no more effective than 3.5% solutions. Patients in our colloid group received approximately 4 gm/kg body weight of albumin during resuscitation. Since this dosage was more than twice that of previously demonstrated effective levels, we did not evaluate resuscitation solutions with even higher concentrations of colloid. Based on our current findings, it is

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36. Berson SA, Yalow RS, Schreiber SS, Post J. Tracer experiments with I^{131} labeled human serum albumin: distribution and degradation studies. J Clin Invest 32:746-768, 1953

37. Rothschild MA, Bauman A, Yalow RS, Berson SA. Tissue distribution of I^{131} labeled human serum albumin following intravenous administration. J Clin Invest 34:1354-1358, 1955

38. Evans EI, Purnell OJ, Robinett PW, Batchelor A, et al. Fluid and electrolyte requirements in severe burns. Ann Surg 135:804-817, 1952

39. Dawidson I, Eriksson B, Gelin L-E, Soderberg R. Oxygen consumption and recovery from surgical shock in rats: a comparison of the efficacy of different plasma substitutes. Crit Care Med 7:460-465, 1979

entirely possible that the use of higher concentrations of albumin may lead to even more pronounced changes in lung water. Although the number of patients in each treatment group is insufficient for statistical analysis at this time, the raw mortality data suggests that the addition of colloid to crystalloid resuscitation solutions may have later deleterious effects. When utilized according to the above described resuscitation guidelines, crystalloid solutions appear to be the preferred fluid for the treatment of acutely burned patients.

PRESENTATIONS

Goodwin CW: Randomized trial of efficacy of crystalloid and colloid resuscitation on hemodynamic response and lung water following thermal injection. To be presented at 1982 Southern Surgical Association Meeting, Palm Beach, Florida, 6 December 1982.

Table 1. Patient Characteristics

	Colloid	Crystalloid
Patients	40	39
Age (years)	28±7	28±8
TBSB (%)	53±17	48±12
Resuscitation (ml/kg/% burn)	2.98 ± 1.10	3.81* ± 1.48

mean ± SD: *p<0.01; TBSB - total body surface burn

Table 2. Left Ventricular Volumes During Postburn Resuscitation

Time Period (hr)	Treatment	Thermodilution CI	ECHO CI	EDVI	SI
0-12	Colloid	3.18 ± .25	3.05 ± .43	42 ± 6	32 ± 5
	Crystalloid	2.59 ± .16	3.11 ± .21	43 ± 3	34 ± 2
12-24	Colloid	3.97 ± .22	4.67 ± .27	56 ± 3	40 ± 2
	Crystalloid	2.14 ± .12*	2.75 ± .25*	36 ± 4*	27 ± 2*
24-48	Colloid	4.17 ± .62	4.42 ± .13	52 ± 3	39 ± 2
	Crystalloid	3.74 ± .45	4.03 ± .40	51 ± 4	37 ± 3

Mean ± SEM: *$p<0.01$ colloid vs crystalloid; CI - cardiac index; EDVI - end diastolic volume index; SI - stroke index; normal: thermodilution CI - 3.60 ± .02 L/min/m^2; ECHO CI = 3.40 ± .04 L/min/m^2; EDVI = 60 ± 3 ml/m^2; SI = 44 ± 2 ml/cycle/m^2

Table 3. Sequential Changes in Lung Water and Cardiac Index Following Thermal Injury

	Treatment	Postburn Day 0.5	1.0	1.5	2.0	2.5	3.0	5.0	7.0
Lung Water (ml/ml)	Colloid	.130 ±.007	.125 ±.005	.120 ±.005	.123 ±.006	.141 ±.009	.145 ±.009	.167 ±.011	.173 ±.015
	Crystalloid	.130 ±.005	.123 ±.004	.124 ±.006	.138 ±.007	.138 ±.008	.140 ±.007	.149 ±.006	.137 ±.011
Cardiac Index ($L/min/m^2$)	Colloid	2.23 ±0.57	2.83 ±0.32	2.41 ±0.29	2.48 ±0.33	2.86 ±0.43	3.60 ±0.33	4.12 ±0.33	5.59 ±0.49
	Crystalloid	2.16 ±0.22	2.42 ±0.14	2.29 ±0.18	2.60 ±0.22	2.90 ±0.22	3.64 ±0.28	4.41 ±0.25	4.99 ±0.40

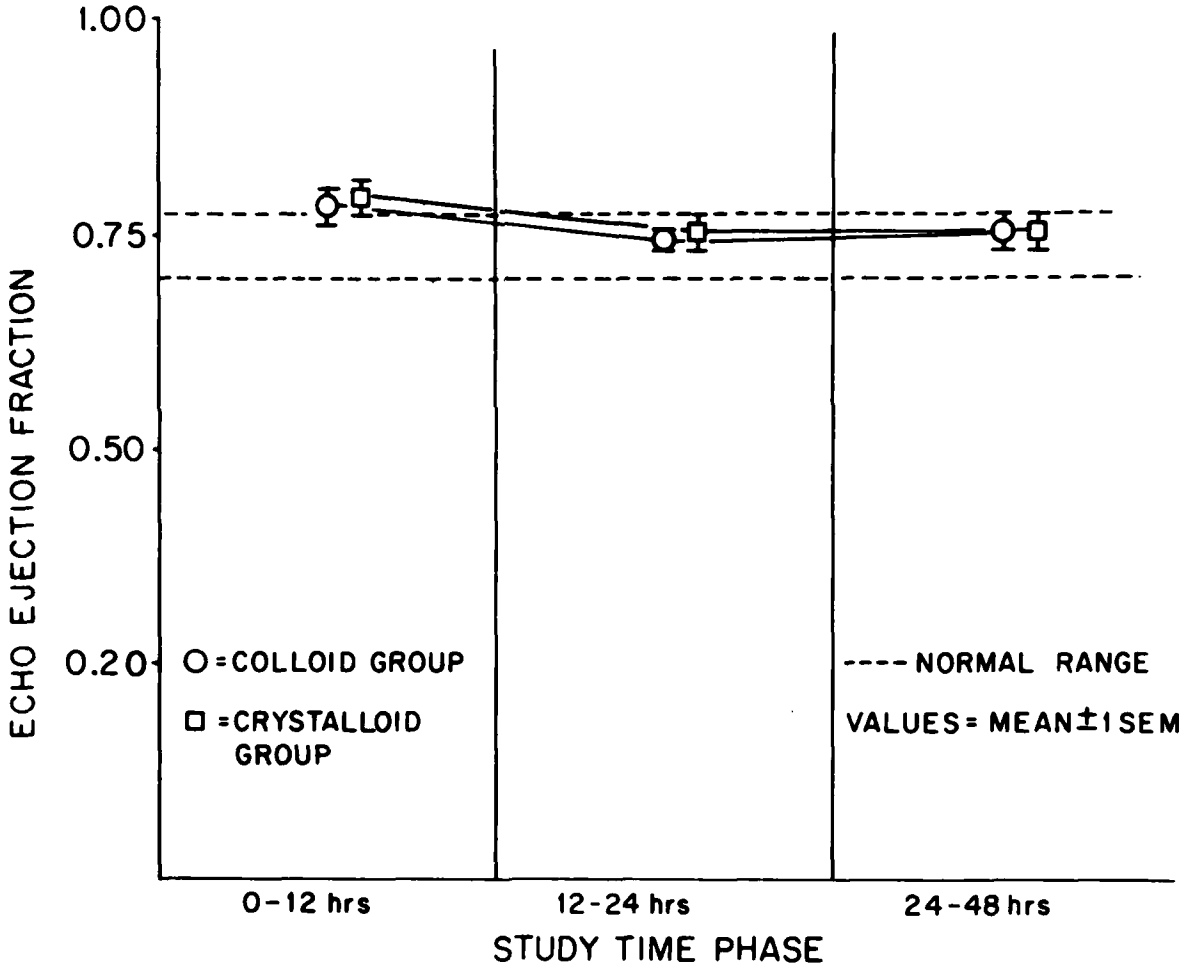

Figure 1. Left ventricular ejection fraction during postburn fluid resuscitation.

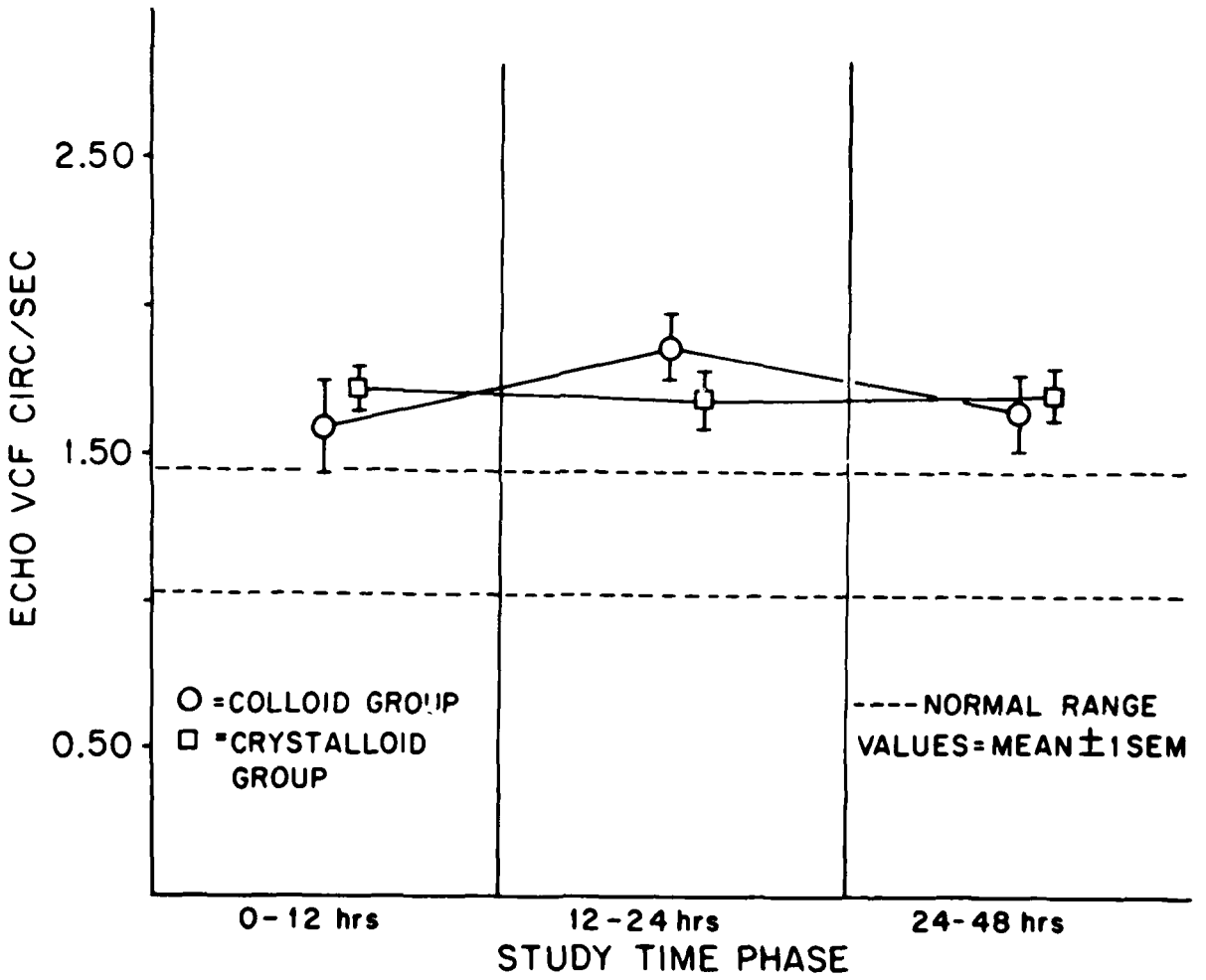

Figure 2. Left ventricular mean rate of internal fiber shortening during postburn fluid resuscitation. The zone above the normal range reflects increased myocardial contractility, while that below reflects decreased contractility.

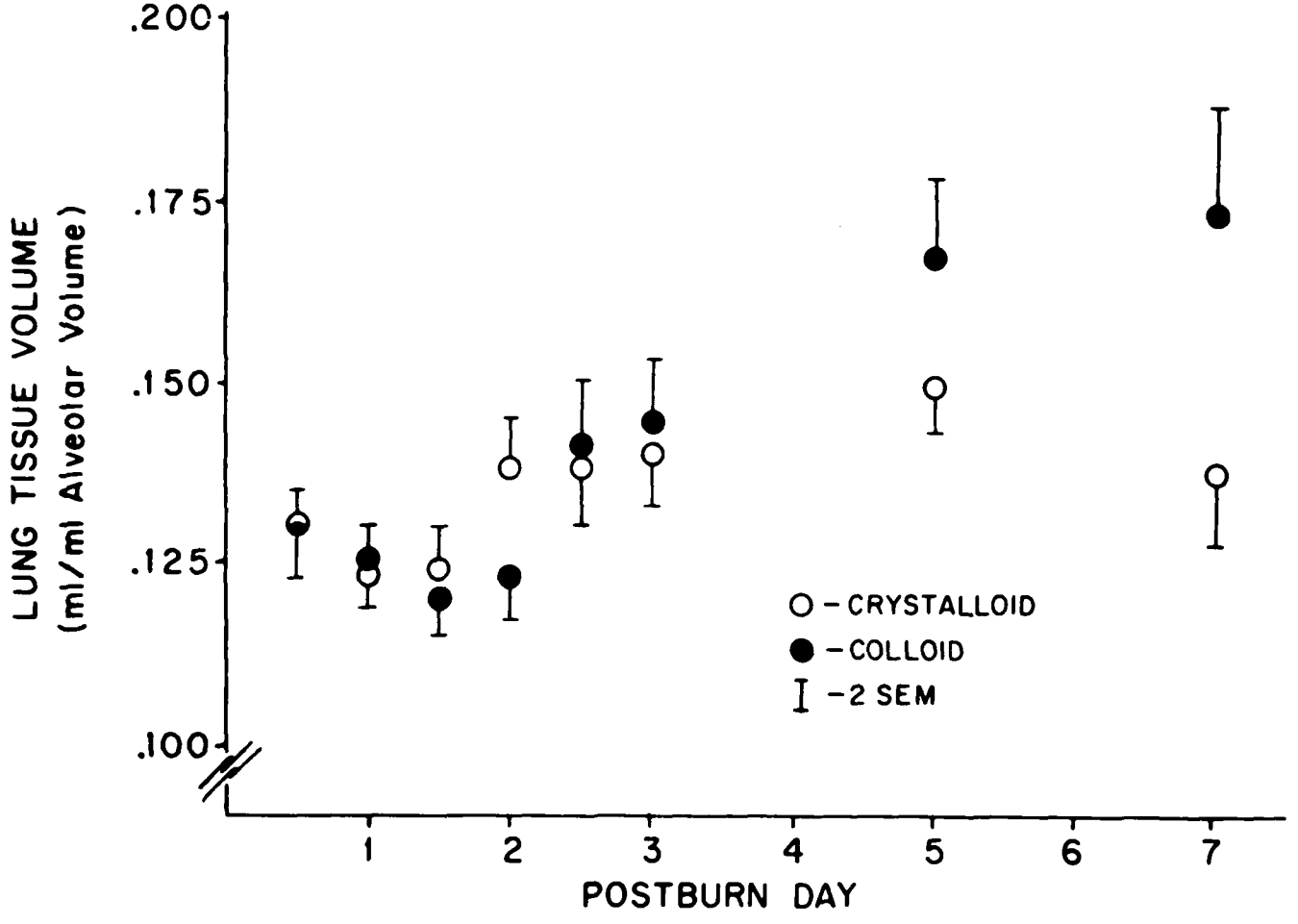

Figure 3. Changes in lung water during the first postburn week for patients resuscitated with either crystalloid or colloid-containing solutions.

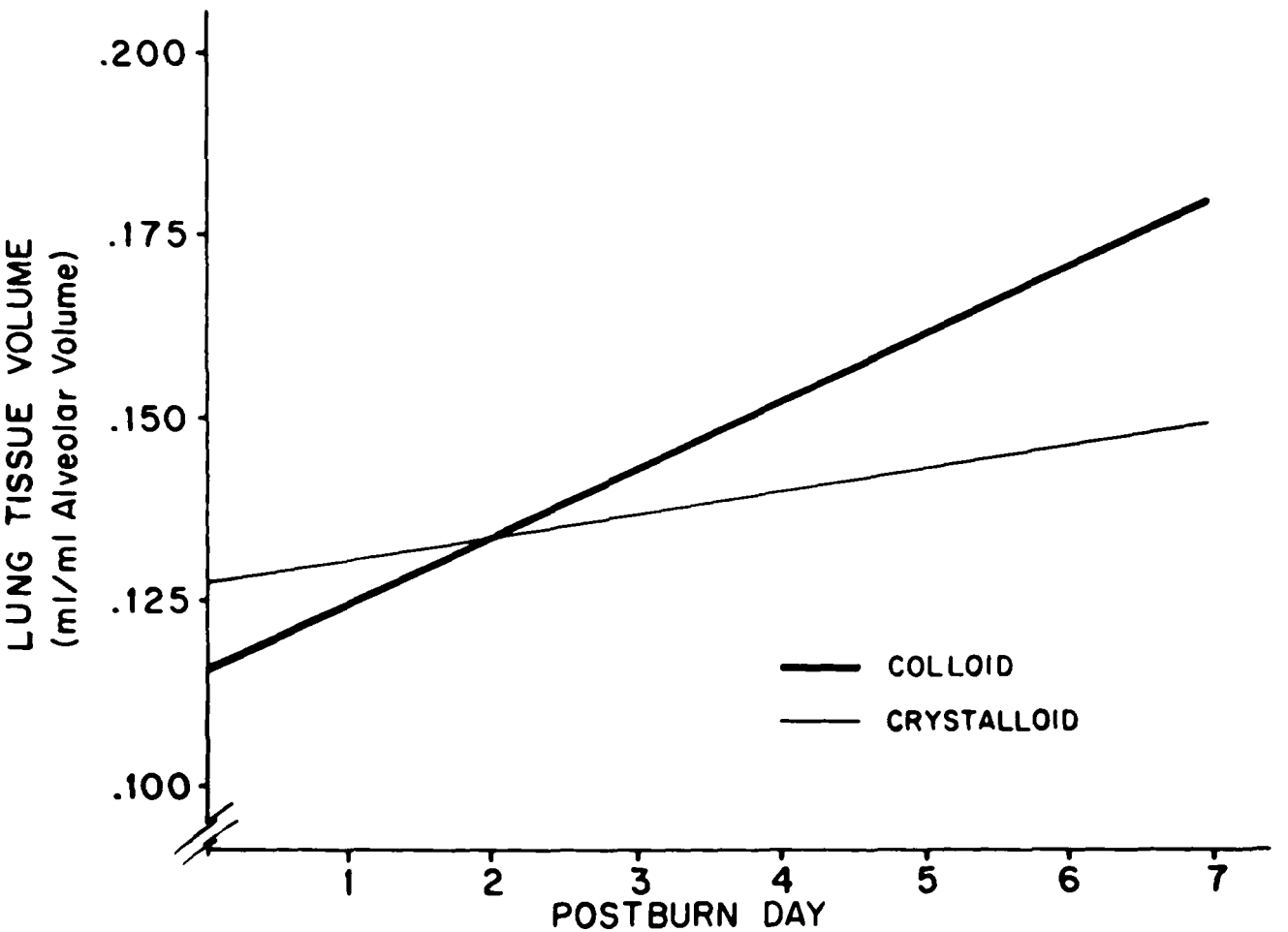

Figure 4. Effect of resuscitation fluid composition when lung water is evaluated as a linear function of time postburn.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6971	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. CHANGE	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AF	162
b. CONTRIBUTING				
c. CONTRIBUTING	STOG 80 - 7.2:5			

11. TITLE (Procede with Security Classification Code)

(U) Evaluation of Burn Wound Care in Troops With Burn Injury

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 10	Cont	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	1.5	60
	CURRENT 83	1.5	70

19a. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

ADDRESS: Fort Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL

NAME: Basil A. Pruitt, Jr., MD, COL, MC

TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

NAME: US Army Institute of Surgical Research
Clinical Division

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TELEPHONE: 512-221-3301

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME: POC: DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Burn Injury; (U) Topical Therapy; (U) Sulfamylon; (U) Wound Excision; (U) 5% Sulfamylon Acetate Solution; (U) Humans; (U) Autografts

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) The continued study of burn wound care is essential if the chances of survival following thermal injury are to be improved. Newer methods under current investigation include an evaluation of the effectiveness of skin substitutes; the influence of burn wound excision on survival and function; the use of subeschar antibiotic clysis to prevent and treat burn wound infection; and, the use of 5% aqueous Sulfamylon soaks.

24. (U) Patients admitted to the U.S. Army Institute of Surgical Research for care following thermal, chemical or electric injury may be, depending on the specific injury, included in studies of these newer modalities of care.

25. (U) 8110 - 8209. The 5% aqueous Sulfamylon soaks were utilized in 146 patients. Eight patients (5.5%) exhibited mild cutaneous atopy. This continued low incidence of side effects coincident with the use of 5% aqueous Sulfamylon along with its apparent clinical effectiveness speaks for its continued use. Standard topical antimicrobial therapy of the burn wound continues to be the sequential application of mafenide acetate and silver sulfadiazine every 12 hours which maximizes the spectrum of antibacterial effectiveness and minimizes the side effects of the respective agents. The indications for burn wound excision continue to be sequential excision limited to 20% of the total body surface

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

at any one procedure in patients with extensive burns; deep dermal hand burns that will not heal within three weeks; removal of tissue with documented wound infection; and debridement of retained non-viable tissue.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

REPORT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: 5% AQUEOUS SULFAMYLON SOAKS USED IN TOPICAL TREATMENT OF BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1981 - 30 September 1982

Investigators:

William F. McManus, M.D., Colonel, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

REPORT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: 5% AQUEOUS SULFAMYLON SOAKS USED IN TOPICAL TREATMENT OF BURNED SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1981-30 September 1982

Investigators: William F. McManus, M.D., Colonel, MC
Basil A. Pruitt, M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

The use of 5% aqueous Sulfamylon dressings in the care of the burn wound has continued to be an efficacious treatment modality throughout this report period. A hundred and forty-six patients were treated with 5% aqueous Sulfamylon dressings employed either for final debridement of a wound or following application of meshed cutaneous autograft to prevent desiccation of tissue exposed in the interstices of such grafts. A 5.5% incidence of skin rash (atopy) was noted as the only adverse reaction. The clinical results achieved by the use of 5% aqueous Sulfamylon solution support its continued use.

Burn injury
Topical therapy
5% Sulfamylon acetate solution
Humans

EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: 5% AQUEOUS SULFAMYLON SOAKS USED IN TOPICAL TREATMENT OF BURNED SOLDIERS

During the reporting period of 1 October 1981 through 30 September 1982, evaluation of 5% Sulfamylon acetate solution for topical treatment of the burn wound has continued at this Institute and involved its use in 146 (68%) of the 215 patients admitted to the U.S. Army Institute of Surgical Research. During this period 208 split thickness autograft procedures were performed in 118 patients; 5% aqueous Sulfamylon soaked dressings were used in conjunction with the skin autografting procedures in 105 patients. The 5% Sulfamylon acetate soaked dressings are used as wet to dry dressings to debride nonviable tissue elements in preparation for split thickness autograft procedures or as continuous wet dressings to protect freshly excised wounds that are not autografted. In addition when meshed cutaneous autografts are applied dressings are soaked with 5% Sulfamylon acetate to decrease the rate of bacterial growth and to prevent desiccation of tissue exposed in the interstices of such grafts.

Eight patients (5.5%) demonstrated allergic reactions (atopy) coincident with the use of 5% aqueous Sulfamylon solution and these eight patients demonstrated rapid resolution of the atopic reaction following administration of an antihistamine and/or discontinuation of the 5% aqueous Sulfamylon soaked dressings. Saline or other aqueous topical antimicrobial agents were substituted once 5% aqueous soaked Sulfamylon dressings were discontinued and no other adverse reactions were noted in this group of patients.

The continued use of 5% aqueous Sulfamylon acetate dressings has been efficacious both in the preparation of the burn wound for cutaneous autografting and in the prevention of desiccation of ungrafted granulation tissue. This efficacy and the low incidence of adverse side effects speak for continued use of this solution.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0G 6970	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. CHANGE	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AF	163
b. CONTRIBUTING				
c. ~~XXXXXXXX~~	STOG 80 - 7.2:5			

11. TITLE (Procede with Security Classification Code)*

(U) Studies of The Neuroendocrine Abnormalities in Burn Injury

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 10	Cont	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	1.7	93
FISCAL YEAR CURRENT 83	1.7	85

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Institute of Surgical Research

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ASSOCIATE INVESTIGATORS

NAME:

NAME: POC: DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Pineal; (U) Hypothalamus; (U) Thyroid; (U) Indoles; (U) Catecholamines; (U) Laboratory Animal; (U) Human Volunteer

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) To determine the hormonal abnormalities in burned soldiers.

24. (U) To measure hormonal concentrations after burn injury under conditions in which other factors known to influence the hormones are quantified or controlled and assess the physiologic effects of the hormones.

25. (U) 8110 - 8209. The hypermetabolism of burn injury correlates better with elevated resting plasma concentrations of catecholamines (best with norepinephrine) than with the elevated cortisol; it exists despite sometimes low concentrations of thyroid hormones, including free concentrations; and does not change with sufficient administration of T_3 to raise plasma T_3 to high in the normal range. Thus, control of metabolism after major burns in humans becomes independent of the thyroid axis and is taken over by the sympathetic nervous system. Burn patients are prone to hyponatremia. Low plasma tonicity was accompanied by elevated ADH, and plasma ADH and urinary tonicity were lowered by further dilution of plasma. Low-normal BUN, normal blood pressure, appreciable Na^+ excretion, and peripheral edema excluded gross volume depletion. Thus, the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) occurs in burned soldiers, results from a reset osmostat, and requires restriction of free water. A hamster model for burns is being developed. After a burn of 24% body surface, there occurs a weight loss of 6% (regained by 18 days) and a low total plasma T_4 which lasts longer and includes suppressed free T_4 at 14 days.

*Available to contractors upon originator's approval

DD FORM 1 MAR 88 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1, 1 MAR 88 (FOR ARMY USE) ARE OBSOLETE

Though pinealectomy did not prevent the weight loss or the low T_4, the pineal may be involved in the neuroendocrine response to burns, because daytime (though not nighttime) pineal melatonin content is reduced in hamsters with this relatively small burn size.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: STUDIES OF NEUROENDOCRINE ABNORMALITIES IN BURN INJURY: I. THYROID HORMONES IN A HAMSTER MODEL WITH ACTIVATED PINEALS OR MELATONIN TREATMENT

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1981 - 30 September 1982

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Reports Control Symbol MEDDH-288(R1)

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ABSTRACT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: STUDIES OF NEUROENDOCRINE ABNORMALITIES IN BURN INJURY: I. THYROID HORMONES IN A HAMSTER MODEL WITH ACTIVATED PINEALS OR MELATONIN TREATMENT

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Blinding resulted in gonadal and prostatic atrophy and reduced plasma thyroxine (T_4), free T_4 index (FT_4I) and reverse triiodothyronine (rT_3) levels in adult male hamsters housed in light-to-dark, 14:10 h. Similar effects were seen after daily evening injections of 25 µg melatonin. Pinealectomy prevented the effects of blinding or melatonin injections. There were no pineal or melatonin-induced decrements in T_3 or thyrotrophin (TSH) concentrations. TSH was elevated by blinding in one experiment but not in another, despite suppression of T_4 and FT_4I in both. Orally administered melatonin (approximately 245 µg daily in drinking water through the evening and night) reduced the weight of testes and prostates and slightly lowered plasma T_4 and FT_4I, indicating the effectiveness of melatonin by this route. The capability of the pineal and of melatonin to suppress plasma T_4 is not a result of sex-steroid-induced alteration of plasma binding but is most likely a result of variable suppression of the pituitary-thyroid axis at the level of TSH regulation and also at the level of T_4 secretion and/or metabolism. Reduced rT_3, but not T_3 levels after blinding, may reflect the pineal-induced deficit in T_4 as a substrate for rT_3 formation, altered peripheral conversion of T_4 or altered disposal of thyroid hormones. The ability of the pineal gland and melatonin to suppress the thyroidal and the reproductive axes indicate the need to examine the role of the pineal gland in burned patients who have suppressed thyroidal and gonadal activity.

Thyroxine (T_4)
Free T_4 Index (FT_4I)
Reverse Triiodothyronine (rT_3)
Blinding
Pinealectomy
Melatonin

THYROID HORMONES IN A HAMSTER MODEL WITH ACTIVATED PINEALS OR MELATONIN TREATMENT

INTRODUCTION

Because of suppression of thyroid and reproductive function usually seen in patients after burn injury, we now use an animal model to examine another condition (restricted photic input) that produces these same effects. Urinary (1) and plasma (2) testosterone levels are suppressed in blind men, as are plasma thyroxine (T_4) levels (2). The hamster has provided one of the best animal models to investigate the endocrine sequelae of reduced environmental lighting. It is now clear that the observed suppression of reproductive function (3) and T_4 concentration (4) in light-restricted or blind hamsters results from activation of the pineal gland by light restriction. Small doses (25 µg) of melatonin, a pineal hormone, injected daily late in the light phase of long photoperiods mimics the effect of the activated pineal gland, causing reproductive collapse (3) and low T_4 levels (4). Pineal or melatonin-induced changes in T_4 have not been ascribed to sex-steroid-related alteration in T_4 transport binding; the changes in free thyroxine index (FT_4I) parallel the changes in T_4.

As yet, it is not clear which components of the rather complicated pituitary-thyroid axis are changed by the activated pineal or by melatonin. In the conventional mammalian scheme (5), pituitary thyrotropin (TSH) stimulates predominantly T_4 secretion from the thyroid, and T_4 is converted peripherally to either triiodothyronine (T_3) or reverse T_3 (rT_3). T_3 is more metabolically active than T_4, and rT_3 is considered inactive.

1. Lenau H, Hollwich F, Dieckhues B, and Nierman H: Der Einfluss des Augenlichts auf das männliche Keimdrüsenhormon. Forschz der Fertil 3:136-139, 1976.

2. Hollwich F, Dieckhues B, and Schrameyer B: Die Wirkung des natürlichen und künstilichen Lichtes über das Auge auf den Hormon-und Stoffwechsel-haushalt des Menschen. Klin Mbl Augenheilk 171:98-104, 1977.

3. Reiter RJ, Peterborg LJ, Brainard GC, de los Santos R, Guerra JC, and Dinh DT: The photoperiod and melatonin in the control of the annual cycle of reproduction in hamsters. In Matthews CD, and Seamark RF (Eds.) Pineal Function. Amsterdam: Elsevier/North Holland Biomedical Press, 1981, pp 95-102.

4. Vriend J, Reiter RJ, and Anterson GR: Effects of the pineal and melatonin on thyroid activity of male golden hamsters. Gen Comp Endocrinol 38:189-195, 1979.

5. Robbins J, Rall JE, and Gorden P: The thyroid and iodine metabolism. In Bondy PK, and Rosenberg LE (Eds.) Metabolic Control and Disease. Philadelphia: W.B. Saunders Company, 1980, pp 1325-1425.

MATERIALS AND METHODS

Adult male golden hamsters were housed four or five per clear plastic cage in a cycle of light-to-dark, 14: 10 h (lights off 2100 h). Standard laboratory chow and tap water were available ad libitum. When the animals weighed approximately 100 g, treatment groups of 8-10 hamsters each were delineated by the surgical procedures performed and/or the other treatment regimens initiated in three experiments. At the end of each experiment, animals were sacrificed by decapitation between 0900 and 1100 h alternately from each group to avoid a systematic influence of time of sacrifice.

In Experiment 1, control sham pinealectomy (CON), pinealectomy (PX), sham pinealectomy plus blinding by removal of both eyes (BL) and combined blinding and pinealectomy (BLPX) were performed. After ten weeks, the animals were weighed and sacrificed, testes and prostates excised and weighed, and trunk blood collected in heparinized plastic tubes for later assay of T_4, T_3, T_3 uptake (T_3U), rT_3 and TSH.

In Experiment 2, four groups received the same surgical procedures as those in Experiment 1 and were not treated with melatonin. Two additional groups were injected subcutaneously with 25 µg melatonin in 100 µl saline daily at 1600-1800 h. One of these two groups was sham pinealectomized (MELsc), and the other was pinealectomized (MELscPX). Ten weeks later at sacrifice, animals, testes and prostates were weighed, and blood was collected in plain plastic tubes for later assay of T_4, T_3, rT_3 and TSH.

In Experiment 3, two unoperated groups (ten hamsters each) received tap water to drink, 100 ml/cage of five hamsters replaced daily and available only between 1600 and 0730 h, beginning 5 h before the onset of darkness. One group (MELpo) received melatonin, 3.1 mg in 100 µl ethanol/100 ml drinking water. The ethanolic stock melatonin solution contained a drop of McCormick green food coloring to help possibly retard photo-oxidation of melatonin. The solution was prepared once, sampled daily and kept in a light-proof glass vial at room temperature in the animal quarters for the duration of the experiment. After adding melatonin or diluent, two drops of green food coloring was also added to the drinking water. Total volume consumed in each cage per evening-night (1600-0730 h) was measured on six occasions from the beginning to the end of the experiment, and the average dose of melatonin was calculated. After eight weeks of treatment, animals and reproductive organs were weighed at sacrifice and trunk blood collected into heparinized plastic tubes for later assay of T_4, T_3, T_3U and TSH.

T_4, T_3 (kits from Diagnostic Products), rT_3 (kits from Serono) and TSH (reagents kindly provided by NIAMDD Rat Pituitary Distribution Program, hamster plasma parallel to RP-1 standard) were measured by radioimmunoassay. Free T_4 index (FT_4I) and free T_3 index (FT_3I)

were the product of the T_3U (kits from Diagnostic Products) and the T_4 or T_3 respectively. Statistical analysis was performed using analysis of variance, followed by the Newman-Keuls test between specific groups if indicated. For Experiment 3 (two groups), the t test, and in one case rectilinear regression and the z test for difference between independent correlations (6) were used.

RESULTS

In Experiment 1 (Fig. 1), compared to CON values, testes and prostates were small in BL but normal (significantly different from those in BL) in BLPX. T_4 and FT_4I were reduced in BL but above control level in BLPX; rT_3, reduced in BL, was normal in BLPX. T_3, FT_3I and TSH showed no differences among the groups except for elevated FT_3I in BL.

FIGURE 1.

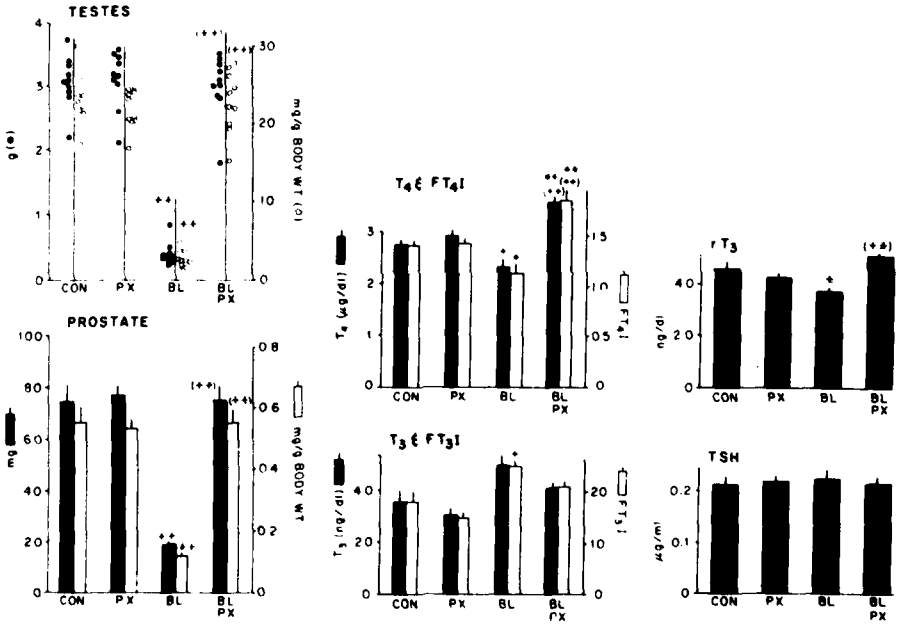

Fig. 1. Experiment 1: Reproductive indices and plasma thyroid hormones 10 weeks after sham pinealectomy (CON), pinealectomy (PX) and/or blinding (BL). Significance symbols (*$p < 0.05$; **$p < 0.01$) denote comparison vs. CON (without parentheses) or vs. BL (with parentheses).

6. Bruning JL, and Kintz BL: Computational Handbook of Statistics. Glenview, Illinois: Foresman and Company, 1977, pp 214-215.

In Experiment 2 (Fig. 2), reproductive organ weights and T_4 were suppressed in BL and MELsc but normal in BLPX and MELscPX. No difference in T_3 among groups was detected. Compared with values in CON, rT_3 was reduced in BL and in MELsc but normal in BLPX. TSH was elevated in BL and normal in BLPX, with no discernable effect of melatonin.

FIGURE 2.

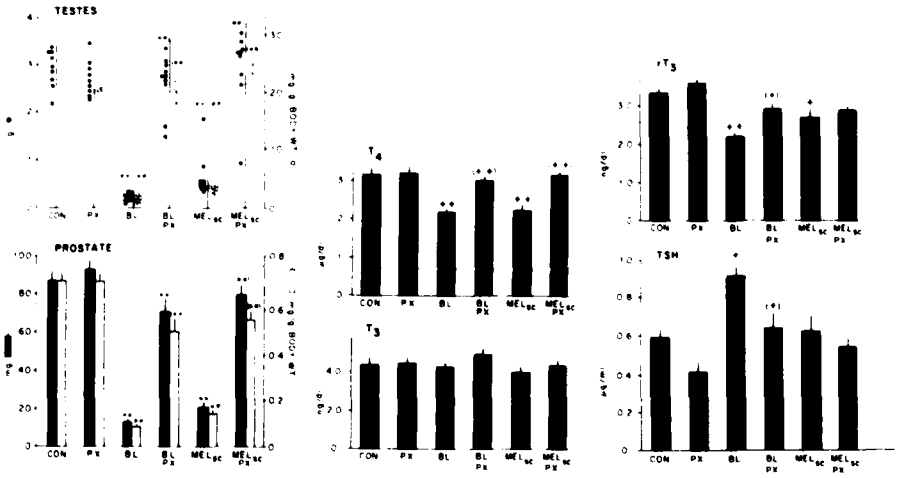

Fig. 2. Experiment 2: Reproductive indices and serum thyroid hormones 10 weeks after sham pinealectomy (CON), pinealectomy (PX), blinding (BL), daily evening injections of 25 µg melatonin subcutaneously (MELsc), or the indicated combinations. Significance symbols (*$p < 0.05$; **$p < 0.01$) denote comparison vs. CON (without parentheses). Parentheses indicate comparison vs. BL for BLPX and vs. MELsc for MELscPX.

In Experiment 3 (Fig. 3), average water intake/hamster/evening-night (1600-0730 h) was 6.9 ml for CON and 7.6 ml for MELpo at the start of the experiment and 7.8 ml for CON and 7.8 ml for MELpo at the end. Mean water intake indicated an average daily dose of melatonin in MELpo of 245 µg/hamster. Testes and prostates were smaller in MELpo compared to those in CON. There was a tendency toward lower T_4 and FT_4I in MELpo (t test not significant). However, FT_4I was significantly correlated with testicular weight in a fashion not significantly different (z test) from the correlation using data from BL and BLPX groups of Experiment 1. No differences were seen between groups for T_3, FT_3I or TSH.

FIGURE 3.

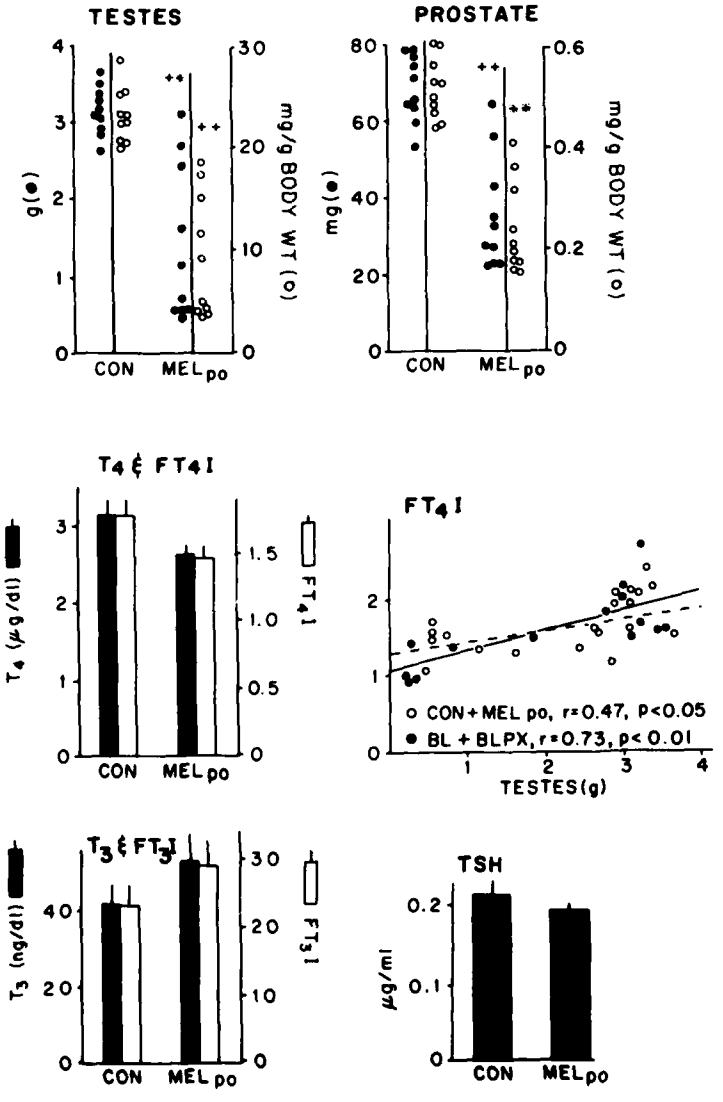

Fig 3. Experiment 3: Reproductive indices and plasma thyroid hormones in hamsters after receiving diluent (CON) or melatonin approximately 245 µg/evening-night (MELpo) for 8 weeks in their drinking water (**$p < 0.01$). For the panel showing the rectilinear regressions, the BL and BLPX data from Experiment 1) show a correlation not statistically different from that using CON and MELpo.

DISCUSSION

The suppressive effects of the pineal gland activated by blinding, and of MELsc in hamsters with intact pineals, on reproductive variables (3) and on circulating T_4 concentration (4) have been confirmed. Although Vriend and Reiter (7) found some effect of 25 µg melatonin in pinealectomized hamsters on T_4 levels (though an attenuated effect), our results (Fig. 2) more clearly suggest pineal dependence of the T_4 suppression due to this dose given over about the same length of time. Although it is not yet known why the presence of the pineal is necessary for the observed melatonin-induced suppression of the gonads and of T_4 levels, one might hypothesize that the injected dose synergizes with the melatonin produced by the pineal during the night. Thus, 25 µg melatonin given early in the light phase was ineffective in suppressing the reproductive system (3) or T_4 levels (8).

We observed no consistent response of T_3 or TSH to blinding or melatonin injection, although in one experiment (Fig. 2), TSH was elevated in BL. Consideration of other reports of suppression of T_3 and TSH after blinding (9) or after melatonin injections of 25 µg late in the light phase (10) allows the conclusion that a pineal effect on T_3 and TSH is a variable response and may be determined by factors not yet understood. However, so far as we know, the response of T_4 and FT_4I to the activated pineal and to melatonin is a consistent one (4,8).

One can envision opposing but variably balanced effects on TSH secretion exerted (a) by an inhibitory action of the pineal at or above the level of the pituitary and (b) by a stimulatory action of low T_4 (reduced negative feedback) from an inhibitory effect of the pineal on the thyroid gland or from accelerated T_4 disposal. Both influences appear to be operative but with a relative intensity that varies among experiments. TSH levels were low in spite of reduced thyroid hormone levels in blind (9) or melatonin treated (10) hamsters, and TSH was not elevated in spite of reduced T_4 in BL (Fig. 1) and in MELsc (Fig. 2), indicating suppression of TSH. On the other hand, suppression of T_4 without suppression of TSH levels in BL (Fig. 1) or MELsc (Fig. 2) and low T_4 in spite of elevated TSH levels in BL (Fig. 2) indicate the

7. Vriend J, and Reiter RJ: Free thyroxine index in normal, melatonin-treated and blind hamsters. Horm Metab Res 9:231-234, 1977.

8. Vriend J, and Reiter RJ: Effects of melatonin and the pineal gland on thyroid physiology of female hamsters. 11th Ann Meeting Soc for Neurosci, Abstracts, Vol 7, p 716, 1981.

9. Johnson LY, Vaughan MK, and Reiter RJ: Effects of blinding and afternoon melatonin injection on parameters of thyroid function in the male golden hamster (Mesocricetus auratus). 11th Ann Meeting, Soc for Neurosci, Abstracts, Vol 7, p 717, 1981.

10. Vaughan MK, Richardson BA, Johnson LY, King TS, Petterborg, LJ, and Reiter RJ: Effects of thyroid-inhibitory and counter-inhibitory afternoon doses of melatonin on T_3 uptake and plasma levels of TSH, T_4, and T_3 in female hamsters maintained under long or short photoperiod. 11th Ann Meeting, Soc for Neurosci, Abstracts, Vol 7, p 717, 1981.

capability of the pineal and melatonin to suppress thyroid secretion directly or accelerate T_4 disposal. What determines whether the inhibitory influence is exerted predominantly at the level of TSH secretion, on the one hand, or at the level of the thyroid or perhaps on thyroid hormone degradation, on the other hand, is not yet understood. Study of thyroid hormone kinetics will be necessary to determine whether accelerated T_4 disposal contributes to the suppression of circulating T_4 concentration observed under the influence of the pineal.

Our finding of reduced circulating rT_3 concentration in BL, which was repeatable (figs. 1 and 2), may reflect the deficit in T_4 as the substrate for rT_3 formation, shunting of T_4 toward T_3 at the expense of rT_3 formation (thereby preventing a fall in T_3 levels) primary inhibition of rT_3 formation or accelerated rT_3 degradation, or a combination of these. Studies of the kinetics of T_4, T_3 and rT_3 will be necessary to determine if there is an effect of the pineal on peripheral conversion of thyroid hormones.

The present studies document that melatonin administered orally can be effective in suppressing the reproductive system (Fig. 3). Though the suppression of T_4 and FT_4I was not statistically significant by the t test, there was a significant correlation of FT_4I with testicular weight which was not statistically different from the correlation of FT_4I and testicular weight observed in blinded hamsters with and without pinealectomy (Fig. 3). This suggests the possibility of a weak effect of oral melatonin on the T_4 levels. The apparently weaker effect of oral compared to subcutaneous administration of melatonin on reproductive variables (some overlap of CON and MELpo testicular and prostatic weights not corrected for body weight) and on T_4 may have resulted from the choice of an oral dose that was not optimal, unequal dosing of hamsters in a given cage, or greater hepatic degradation of melatonin.

Because both the reproductive system and T_4 levels change in hamsters with melatonin treatment or with pineals activated by blinding, it is necessary to consider whether changes in T_4 levels are simply a result of altered binding of T_4 in plasma secondary to changes in sex steroid levels. This is not the case because: (a) androgens tend to decrease levels of thyroxine binding globulins (5) such that a possible increased thyroxine binding globulin in androgen deficiency might be expected to elevate rather than suppress T_4 levels; (b) blinding or melatonin treatment suppresses the reproductive system also in female hamsters and reduces T_4 levels whether or not they are ovariectomized (8); (c) orchiectomy did not suppress T_4 levels or FT_4I in male hamsters (4); (d) in some experiments there has been no suppression of T_3 levels (figs. 1 and 2); (e) in one experiment (Fig. 2) blind animals had elevated TSH, suggesting physiologic significance of reduced free T_4 in that case; and (f), blinding- or melatonin-induced reduction in T_4 has always been associated with a parallel reduction in FT_4I (4,7,9, 10), the latter correcting for possible changes in plasma T_4 binding sites. (5).

The pineal gland and melatonin are capable of suppressing both the reproductive system and circulating T_4 and rT_3 concentrations. The effect on T_4 levels is not the result of a change in T_4 plasma binding related to reduced sex steroid levels. Rather, it is a combination of suppression at or above the level of the pituitary together with effects either directly on thyroidal secretion or on peripheral metabolism of T_4. Since burn injury of humans results in low T_4 and low testosterone, the question of whether the pineal gland is the mediator of these responses also in burn injury is currently being addressed using this animal model.

PRESENTATIONS/PUBLICATIONS

Vaughan GM, Vaughan MK, Seraile LG, and Reiter RJ: Thyroid hormones in a hamster model with activated pineals or melatonin treatment. Presented to the Symposium on The Pineal and Its Hormones, January 2-4, 1982, and published by Alan R. Liss, Inc., New York, New York, pp 187-196, 1982.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S16772A874-00, APPLIED RESEARCH

PROJECT TITLE: STUDIES OF NEUROENDOCRINE ABNORMALITIES IN BURN INJURY: II. THYROIDAL, REPRODUCTIVE AND PINEAL FUNCTION IN A HAMSTER BURN MODEL

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Hamsters may provide a model for the neuroendocrine responses of burned patients, because like humans, they respond to a burn with 1) weight loss, 2) reduction in circulating T_4, 3) reduction in free T_4 concentration, 4) reduction in serum binding of T_4, 5) excessive suppression of a commonly used index of free T_4 in proportion to suppression of FT_4, and 6) suppression of plasma testosterone. In addition, the largest burn practicable in these animals (23%), used in all experiments, produced a lowering of daytime but not nighttime melatonin content of the pineal gland. In another hamster model (blinding), suppression of T_4, free T_4 index, free T_4, and reproductive organ weights is pineal mediated. However, in short-term pinealectomy experiments, the burn-induced reduction in T_4 and testosterone is not pineal-mediated. The pineal may retard the early postburn reduction in plasma testosterone concentration. However, the burn-induced reduction in testicular weight is mediated by the pineal gland. These studies are the first to provide evidence for a role of the pineal gland in the neuroendocrine response to trauma.

Thyroxine (T_4)
Free T_4 Index (FT_4I)
Blinding
Pinealectomy
Melatonin

STUDIES OF NEUROENDOCRINE ABNORMALITIES IN BURN INJURY: II. THYROIDAL, REPRODUCTIVE AND PINEAL FUNCTION IN A HAMSTER BURN MODEL

INTRODUCTION

Burn injury in humans results in suppression of the thyroid and gonadal axes (1-4). Restriction of photoperiod or blindness has these same endocrine effects in humans and animals, and these effects are mediated by the pineal gland in animals (1). This interesting combination of observations raises several fundamental questions, including whether burns in an animal model produce the same endocrine effects as in humans and whether such endocrine effects are mediated by the pineal gland. Most of the work on the pineal dependency of the endocrine responses to interrupted visual input has been done in hamsters. Pineal-dependent changes are more readily produced in this species than in the laboratory rat which has been bred for many more generations in laboratory environments in which survival does not depend on detecting and responding to seasonal occurrence of restricted (winter) photoperiod. Although blind hamsters have suppressed thyroxine (T_4) concentrations and free T_4 index (FT_4I), it has not been determined whether they have suppressed free T_4 (FT_4) concentrations as determined by dialysis. Thus, we have assessed the ability of the pineal (responding to blindness) to alter FT_4 in hamsters. Further, we have investigated the male hamster as a burn model, recording the alterations in body weight, concentrations of total thyroid hormones, free serum thyroxine concentration, and reproductive variables. Finally, we have obtained initial data suggesting pineal involvement in the neuroendocrine response to injury.

METHODS

Male golden hamsters, Mesocricetus auratus, were purchased from the Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts, at about 90 g body weight (young adults) and maintained in our animal quarters in a light/dark environment of 14/10 h with lights on at 0700 h and given standard laboratory chow and tap water ad libitum. In all

1. Vaughan, GM, Vaughan MK, Seraile LG, and Reiter RJ: Studies of neuroendocrine abnormalities in burn injury: I. Thyroid hormones in a hamster model with activated pineals or melatonin treatment. In U.S. Army Institute of Surgical Research Annual Research Progress Report, 1 October 1981 - 30 September 1982. U.S. Army Research and Development Command, Ft. Detrick, MD.
2. Vaughan GM, and Becker RA: Unpublished observations.
3. Dolocek R: Unpublished observations.
4. Molteni A: Unpublished observations.

experiments, pinealectomy, blinding or burning was carried out when the animals reached about 110 g. Blinding was by bilateral orbital enucleation, pinealectomy by the method of Hoffman and Reiter (5) and burning by a modification of the standard method for rats (6).

Excised skin surface area of unburned 110 g hamsters was measured by planimetry. A burn mold with an exposure window of 27.25 cm^2 would reproducibly retain the hamster with window edges fitting snugly enough to exclude hot water from skin outside the area of the window and also maintain a constant area of skin exposed through the window. Larger windows failed to do this, so that the largest burn (back plus abdomen) that was practical was 23% of body surface area. Therefore, in all the experiments with burns, the total burn size was 23%. Under Na pentobarbital anesthesia (30-35 mg/kg i.p.), the hair was clipped and the animal placed in the burn mold. Exposure to 80°C scalding water for 8 seconds (back), followed by injection of 5 ml physiologic saline i.p., then exposure for 4 seconds (abdomen) resulted in full-thickness thermal injury involving all layers of the epidermis and dermis and occasionally the superficial layers of the panniculus muscle beneath the abdominal skin. Sham-burned hamsters received the entire procedure (including hair clipping) except that they were exposed to water of room temperature. Control hamsters (without the hair clipped and not exposed to water) were used in some experiments.

All animals were housed 4-7 per clear plastic cage with other members of the same group.

All mortality occurred in the first 48 h following an invasive procedure and was restricted to animals that had been anesthetized. Deaths in burned animals usually occurred in the first 12 h after burning. Of pinealectomized hamsters, 1/9 additionally blinded died, and 4/24 with only added sham burning and 3/28 with added burning (Exp. 9) died. Of burn control animals with no anesthesia, i.p. saline, burn or other surgical procedure, 0/10 died, and 1/30 controls with anesthesia and i.p. saline died. In experiments not involving pinealectomy or blinding, 0/209 sham-burned and 8/237 (3.4%) burned hamsters died.

In the experiments listed below, unless otherwise indicated, there were 6-12 hamsters in each treatment group at a particular time of sacrifice. All animals were sacrificed by guillotine decapitation between 0800 and 1100 (unless other times are specified), alternating among groups to avoid a systematic error in variables between groups and related to passage of time during the sacrifice. Body weights were recorded just prior to burning and at sacrifice.

5. Hoffman RA, and Reiter RJ: Rapid pinealectomy in hamsters and other small rodents. Anat Rec 153: 19-22, 1965.

6. Walker HL, and Mason AD Jr: A standard animal burn. J Trauma 8: 1049-1051, 1968.

Analyses. Thyroxine (T_4), triiodothyronine (T_3), reverse T_3 (rT_3), *in vitro* T_3 charcoal uptake (T_3U), and testosterone analyses were performed with kits from Diagnostic Products. Free T_4 index (FT_4I was the product of the T_4 and T_3U. The dialyzable fraction of T_4 (T_4D) was determined at the Nichols Institute, San Juan Capistrano, California, by equilibrium dialysis, and the product of the T_4 and the T_4D is free T_4 concentration (FT_4). Melatonin was measured using the Rollag procedure as modified (7). Melatonin in experiments 4 and 6 was determined by Dr. R.J. Reiter at the University of Texas Health Science Center at San Antonio, and all other melatonin values were determined in this laboratory. All hormone concentrations were determined by radioimmunoassay. Zinc (Zn) was determined by atomic absorption spectrometry under the auspices of Dr. M. Powanda and Mr. Y. Villarreal, Chemistry Branch, of this institute. All assays were performed such that all groups to be compared were represented in alternating positions throughout one assay.

The Student t test was used to compare the means of a variable between two groups. For more than two groups in a comparison, a one-way analysis of variance followed by a Student-Newman-Keuls test (contingent upon F with $p < 0.05$ for the null hypothesis) was used to compare means. To determine if the relationship between two variables differed between two groups, analysis of covariance(ANOCOVA) and multiple linear regression analysis with group as the other independent variable were used. In the ANOCOVA, if no difference in slope between groups was detected ($p < 0.05$), then a difference in group position was tested for significance.

Experiment (Exp) 1. The animals were divided into three groups: sham-pinealectomized (SHPX), blinded and sham-pinealectomized (BL-SHPX), and blind and pinealectomized (BL-PX). Eleven weeks later, the animals were sacrificed by decapitation, body and reproductive organs were weighed, and trunk serum was saved for determination of T_4, T_3U, and T_4D.

Experiment (Exp) 2. Animals were divided into sham-burned and burned groups and sacrificed on postburn days (PBD) 1, 4 and 11. Heparinized trunk plasma was saved for determination of T_4, T_3 and T_3U.

Experiment (Exp) 3. Control, sham-burned and burned hamsters were sacrificed on PBD 1, 3 and 7 and heparinized trunk blood was saved for analysis of T_4, T_3, T_3U, rT_3 and testosterone.

Experiment (Exp) 4. Control, sham-burned and burned animals were decapitated on PBD 6. Heparinized plasma was saved for determination of T_4, T_3, T_3U, rT_3 and testosterone. Pineals were excised and saved ($-70^{\circ}C$) for determination of melatonin. Animals were earmarked so that the sacrifice weight could be matched in the same animal with the pre-burn weight.

7. Vaughan GM, Allen JP, Tullis W, Siler-Khodr TM, De La Peña A, and Sackman JW: Overnight plasma profiles of melatonin and certain adenohypophyseal hormones in men. J Clin Endocrinol Metab 47: 566-571, 1978.

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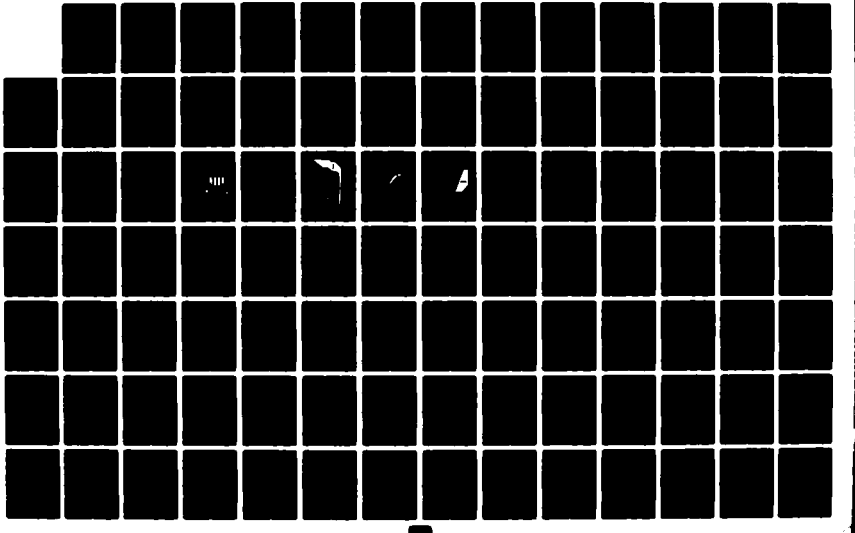

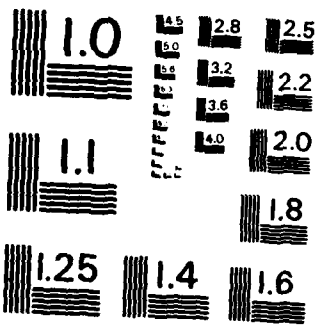

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Experiment (Exp) 5. At the usual time in the morning (0800-1000 h), sham-burned and burned animals were sacrificed on PBD 1 and 13, and at 0400 h on PBD 14. On PBD 7, control, sham-burned and burned hamsters were sacrificed at 0800-1000 h. EDTA plasma from those sacrificed on PBD 7 was saved for assay of T_4, and serum from the PBD 14 sacrifice was saved for T_4 and T_4D analysis. The pineals from all animals in the experiment were saved for melatonin determination.

Experiment (Exp) 6. Sham-burned and burned hamsters were sacrificed on PBD 5, and serum was saved for determination of T_4, T_3U and T_4D. Pineals were taken for assay of melatonin. There were 9 sham-burned and 18 burned animals.

Experiment (Exp) 7. Sham-burned and burned hamsters were sacrificed on PBD 14, 21, 28, 35 and 42. Serum was saved for analysis of T_4, T_3U and T_4D. Pineals were saved for assay of melatonin. Serum variables are available for PBD 14 and 21, and pineal melatonin for PBD 14, 21 and 28. These animals were sacrificed 4-6 hours into the light phase.

Experiment (Exp) 8. Sham-burned and burned hamsters were sacrificed every two hours on PBD 7, completing the first sacrifice just before the onset of darkness at 2000 h and the last sacrifice just before the end of the dark period at 0600 h. All pineals were taken for melatonin assay, and serum at the 0400 h and 0600 h time points was taken for T_4 assay.

Experiment (Exp) 9. Animals were initially divided into sham-pinealectomized (SHPX) and pinealectomized (PX) groups. Two days following these procedures, each of the initial groups was divided into sham-burned (SHBU) and burned (BU) animals by performing these procedures. Further, one-half of each of the resulting groups (SHPX - SHBU, SHPX-BU, PX-SHBU, PX-BU) was sacrificed on PBD 6 and the other half on PBD 14. At sacrifice, there were 9-14 animals in each of the 8 groups. Reproductive organs were weighed, and one testis was saved for determination of Zn. Body weights were recorded and paired with pre-burn weights, utilizing ear markings for identification of animals. Livers were perfused with physiologic saline, and an anterior wedge was resected, weighed and analysed for Zn. Serum was saved for assay of T_4, testosterone, rT_3 and Zn.

RESULTS

Effect of the pineal on T_4.

Exp. 1. Blindness (BL-SHPX) suppressed testicular and prostatic weights whether or not organ weight was corrected for body weight (Fig. 1). T_3U was lower in the BL-PX group than in the other two groups, and there were no differences in T_4D (Table I). Blinding markedly suppressed T_4, FT_4I and FT_4 (Fig. 2). None of the changes

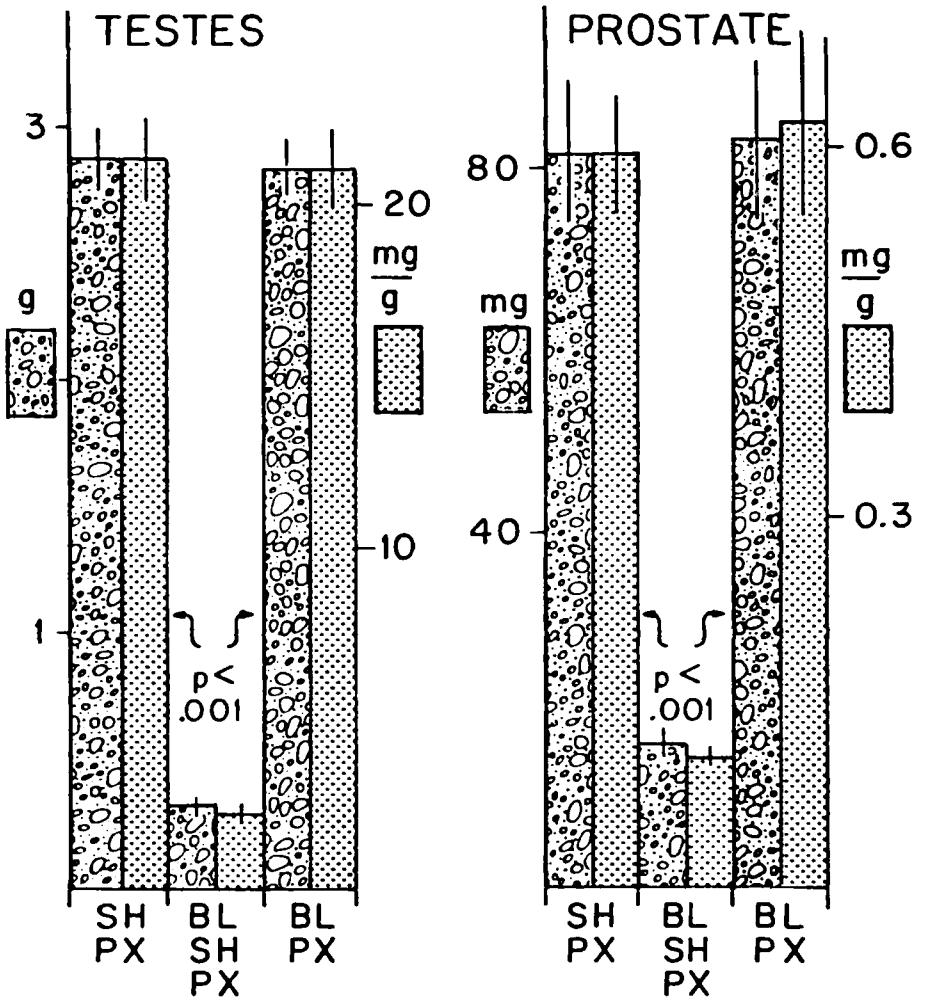

Figure 1. Testicular and prostate weights in Experiment 1, as absolute (g)or relative (mg/g body weight) values 11 weeks after sham pinealectomy (SHPX), blinding (BL) and/or pinealectomy (PX).

Table I. In vitro T_3 uptake (T_3U) and dialyzable fraction of serum T_4 (T_4D) 11 weeks after sham pinealectomy (SHPX), blinding (BL) by orbital enucleation, and/or pinealectomy (PX), in Exp. 1.

	SHPX		BL-SHPX		BL-PX	
	T_3U (%)	T_4D (%)	T_3U (%)	T_4D (%)	T_3U (%)	T_4D (%)
Mean	38.04	0.0638	38.66	0.0581	36.63**	0.0504
SE	0.319	0.004	0.293	0.004	0.404	0.006
n	11	11	12	12	8	8

**$p < 0.01$ vs SHPX and BL-SHPX.

Table II. In vitro T_3 uptake (T_3U) at various postburn days (PBD) in experiments 2 (PBD 1,4,11) and 3 (PBD 1,3,7)

	Sham Control	Sham Burn	Burn	Sham Control	Sham Burn	Burn	Sham Control	Sham Burn	Burn
PBD		1			4			11	
Mean		49.40	50.01		49.32	51.55†		46.54	48.49**
SE		0.172	0.699		0.269	0.330		0.426	0.317
n		5	7		8	7		7	6
PBD		1			3			7	
Mean	42.11	40.37**	41.08§	41.66	39.98†	41.84†	41.39	39.67*	41.22**
SE	0.395	0.295	0.287	0.248	0.275	0.303	0.633	0.326	0.301
n	7	8	8	7	8	8	6	8	10

*$p < 0.05$, **$p < 0.01$, †$p < 0.001$ vs control (for sham burn) or vs sham burn (for burn). §$p < 0.05$ vs control.

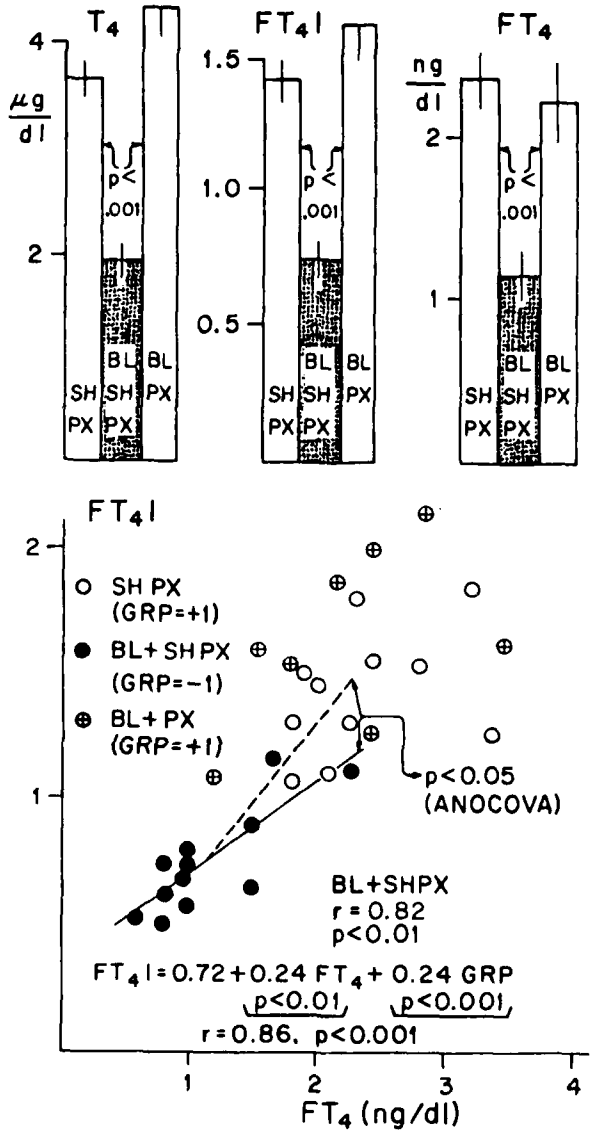

Figure 2. Thyroxine (T_4), free T_4 index (FT_4I) and free T_4 (FT_4) in the hamsters of Experiment 1. The error lines in the upper panel (as in all other graphs) are SE. The regression line (bottom panel) is that for only the BL-SHPX group. The dotted line connects the mean point of the BL-SHPX group with the combined mean of the other two groups and provides the index of comparison for the position test using analysis of covariance (ANOCOVA). For the multiple regression, group (GRP) for each value was assigned a value of +1 or -1 as indicated.

due to blinding was present if also the pineal gland had been removed (BL-PX). FT_4I was correlated with FT_4 among all animals and in the BL-SHPX group alone, but not in the other two groups together (Fig. 2). FT_4I in BL-SHPX was lower than expected for the general relationship of FT_4I to FT_4 as seen by both a multiple regression with group as a variable and an ANOCOVA. SHPX and BL-PX were considered as one group, because (a) both these groups lacked a stimulated pineal gland (visual perception of the long photoperiod and pinealectomy both remove pineal influence) and (b) FT_4I and FT_4 were not different between these groups. In order to minimize the likelihood of ANOCOVA significance for position difference, the combined group with unstimulated pineals was assigned a slope of 0 instead of the calculated value (since within the group the correlation was not significant), the BL-SHPX group's own slope was used instead of the common slope, and for the gruop with unstimulated pineals, the total (instead of the residual) sum of squared deviations was used. The resultant p value was < 0.05. Without the substitutions, p was < 0.001.

Effect of burning on thyroid hormones.

Exp. 2, 3 and 4. Figure 3 shows that T_4 was suppressed in burns compared to shams on PBD 3, 4, 6, 7 and 11, and perhaps on PBD 1. On PBD 3, and to a lesser extent on PBD 7, T_4 was lower in shams than in controls. The same relationships among groups appear in the FT_4I. Suppression of rT_3 in burns was seen on PBD 1, 3, 6 and 7, but suppression in shams was less dramatic or consistent. The large variation of T_3 patterns between experiments precludes a definitive assessment, except that T_3 was lower in burns than in sham burns on PBD 1. In Exp. 4, a weight loss (by PBD 6) was seen only in burns. T_3U was almost always higher in burns, compared to shams (Table II), and in some cases, T_3U was lower in shams than in controls.

Exp. 5. Figure 4 shows that on PBD 7, burns suppressed T_4 measured at the usual time (0800-1000 h)and suppressed T_4 and FT_4 measured at 0400 h on PBD 14, compared to values in animals with sham burn.

Exp. 6 and 7. Figure 5 shows that T_4 was suppressed in burned animals compared to shams at PBD 5, 14 and 21. However, FT_4 was not suppressed in the burn group on PBD 5, whereas it was on PBD 14 and less dramatically so on PBD 21. FT_4I was suppressed in burns relatively more than was the FT_4 at all three time points, as shown by the regression analyses and ANOCOVAs (Fig. 5). Although the regression of FT_4I on FT_4 tended to be positive in most groups, it was negative in the sham burns on PBD 14. Whereas T_3U did not differ between groups, T_4D was elevated in burns at all three time points (Table III).

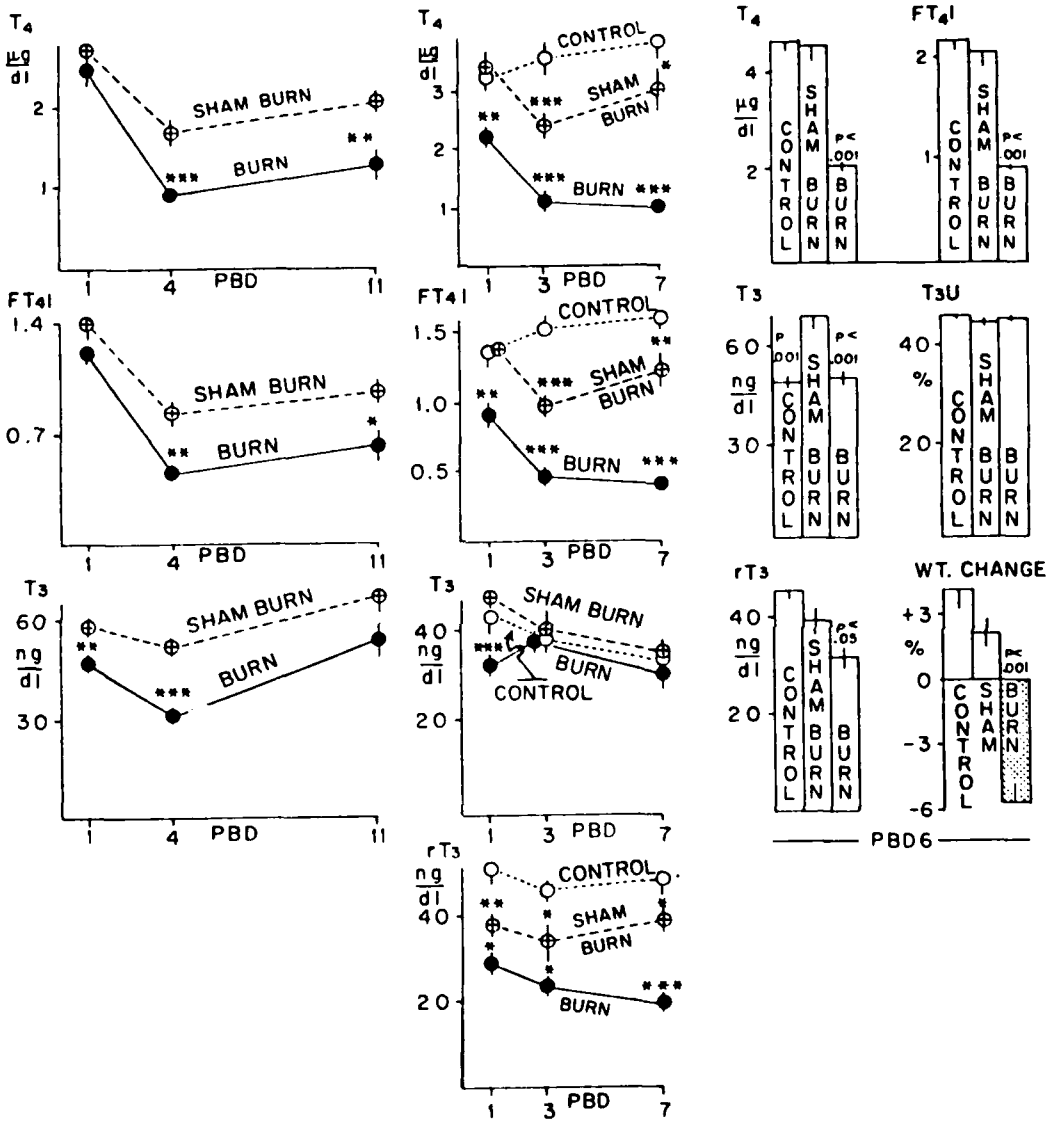

Figure 3. Values for thyroxine (T_4), its free index (FT_4I), triiodothyronine (T_3), reverse T_3 (rT_3) and T_3 uptake (T_3U) in experiments 2 (left panel), 3 (middle panel) and 4 (right panel). Weight change indicates change from pre-burn weight. A p value over a bar compares that bar with the one adjacent to it. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$, comparing burn with sham burn or sham burn with control groups at the postburn day (PBD) indicated.

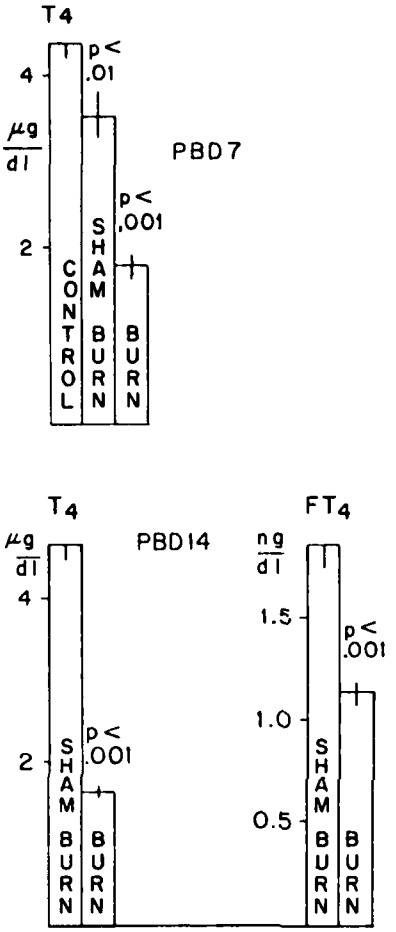

Figure 4. Thyroxine (T_4) and free T_4 (FT_4) on postburn day (PBD) 7 (early light phase) or 14 (dark phase). p values compare a bar with the adjacent one to the left.

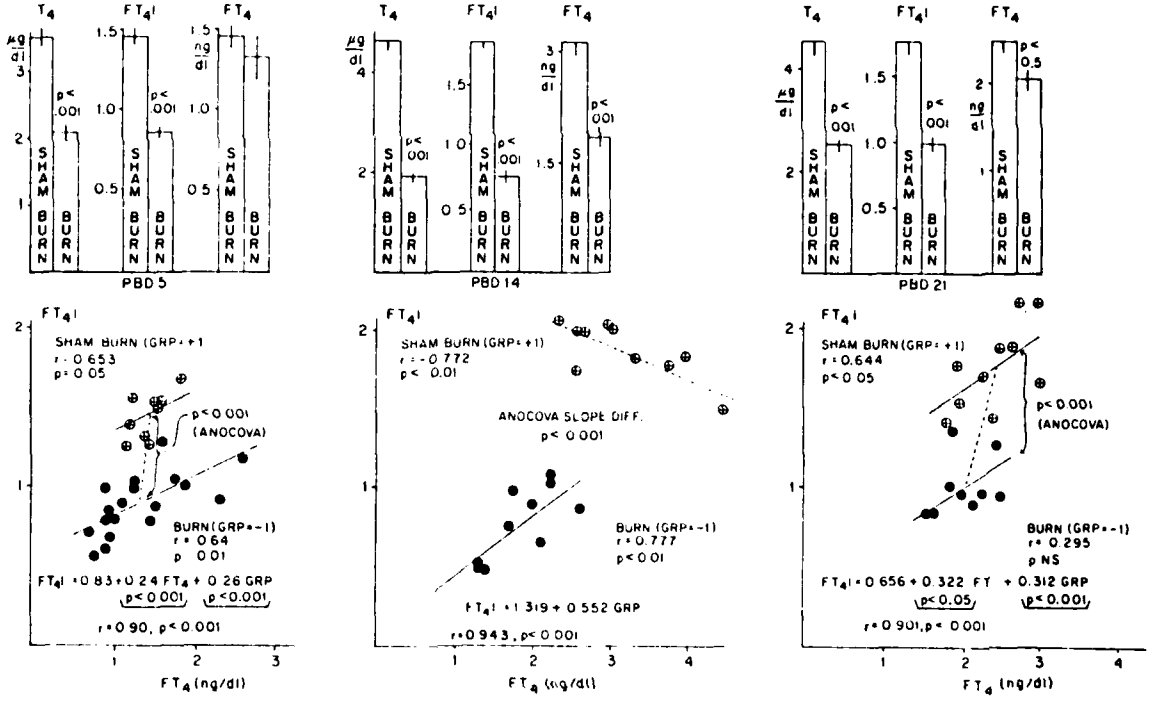

Figure 5. Thyroxine (T_4), its free index (FT_4I) and free concentration (FT_4) in experiments 6 (left panel) and 7 (middle and right panels) on postburn days (PBD) indicated. The regression lines are based on the multiple regression analyses shown above the abscissae, except in the middle panel in which they are based on the individual group linear regressions. ANOCOVA, analysis of covariance.

Table III. In vitro T_3 uptake (T_3U) and dialyzable fraction of serum T_4 (T_4D) at various postburn days (PBD) in experiments 6 (PBD 5) and 7 (PBD 14,21).

	EXPERIMENT 6				EXPERIMENT 7							
	PBD 5				PBD 14				PBD 21			
	SHAM BURN		BURN		SHAM BURN		BURN		SHAM BURN		BURN	
	T_3U (%)	T_4D (%)	T_3U (%)	T_4D (%)	T_3U (%)	T_4D (%)	T_3U (%)	T_4D (%)	T_3U (%)	T_4D (%)	T_3U (%)	T_4D (%)
Mean	41.17	.0411	41.79	.0621†	40.36	.0699	39.62	.1001**	38.87	.0541	39.00	.0804†
SE	.588	.001	.290	.005	.191	.007	.368	.005	.414	.003	.443	.004
n	9	9	18	18	10	10	10	10	10	10	9	9

**$p < 0.01$, †$p < 0.001$ vs SHAM BURN.

Composite results of weight change and T_4 in burns.

Combining all experiments not involving pinealectomy or blinding, Fig. 6 shows that burned hamsters lost about 6% of pre-burn weight (maximal in the second week) and returned to pre-burn weight by about PBD 18. However, suppression of circulating T_4 was much more dramatic and was present from PBD 1-21 (Fig. 6). Data points are means for groups. For body weights, mean sacrifice weight less the pre-burn weight (body weight change), as a percent of the pre-burn weight, was utilized.

Plasma testosterone in burns.

Figure 7 shows that on PBD 1, 3, 6 and 7, mean testosterone concentration was lower in burned hamsters than in shams or controls, and the difference was significant on PBD 3 and 7. On PBD 1, sham values were lower than in controls.

Pineal melatonin in burns.

Although at eight different points from PBD 1-28, mean pineal melatonin in the morning (0800-1000 h) was lowest in the burn groups (Fig. 7), comparing burns and shams, this difference was smallest on PBD 1 and 28, and greatest on PBD 14 at which point pineal melatonin was suppressed to 50% of the sham value. Figure 8 depicts the nocturnal pattern of pineal melatonin. On PBD 7, at the 2000 h time point, still during the light phase, values were significantly lower in burns than in shams, corroborating the suppression of daytime pineal melatonin values in burns noted above. However, the normal nocturnal surge in melatonin was not affected by burning as seen on PBD 7 with 2-hourly values and on PBD 14 with 0400 h values.

Effect of pinealectomy on the response to burning.

Figure 9 shows that pinealectomy two days before sham-burning lowered T_4 at PBD 6 but not at PBD 14 (SHPX-SHBU vs PX-SHBU). However, the dramatic suppression of T_4 in burned hamsters evident at PBD 6 and 14, was unaffected by pinealectomy two days before burning (SHPX-BU vs PX-BU). Testicular weight was unaffected by burning and/or pinealectomy on PBD 6 (Table IV, Fig. 9). However, by PBD 14, the pinealectomized groups had relative sparing of testicular mass reduction. That is, the reduction of body weight gain due to pinealectomy (PX-SHBU vs SHPX-SHBU) was accompanied by slightly higher mean relative testicular weight and, more dramatically, the weight loss due to pinealectomy plus burning (PX-BU vs SHPX-SHBU) was accompanied by significantly higher relative testicular weights (Fig. 9). The reduction in testicular mass by PBD 14 in burns was prevented by pinealectomy (Table IV). On both PBD 6 and 14, both pinealectomy

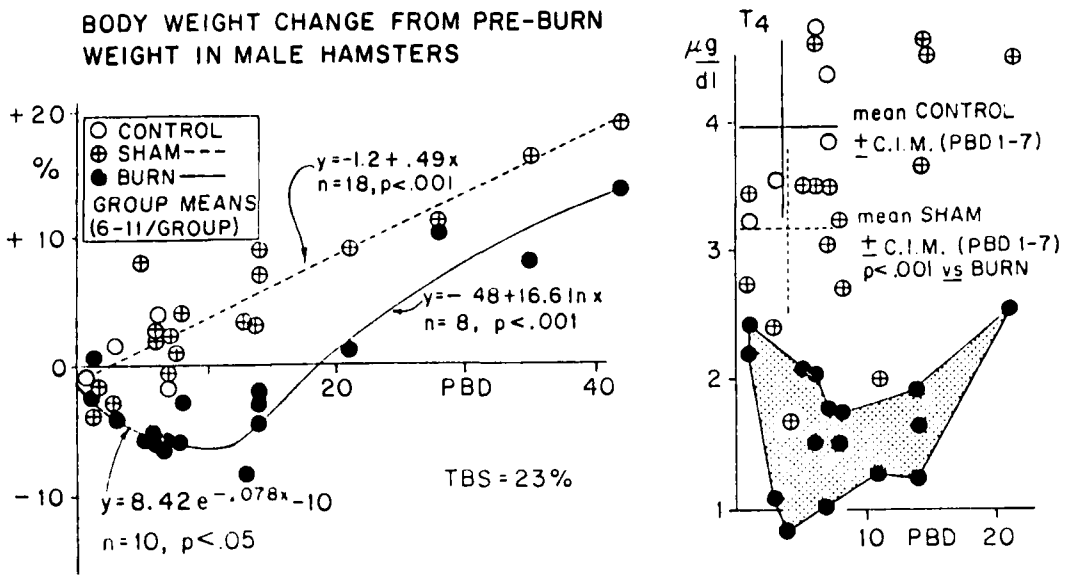

Figure 6. Body weight change and circulating T_4 concentrations at various postburn days (PBD) in hamsters with total burn size (TBS) of 23% body surface. The burn weight curve results from the two regressions with their junction point rounded by hand. For the T_4, C.I.M. indicates the 95% confidence interval of the mean, and the shaded area visually approximates the range of values for the burn groups. In both graphs, each data point is a group mean.

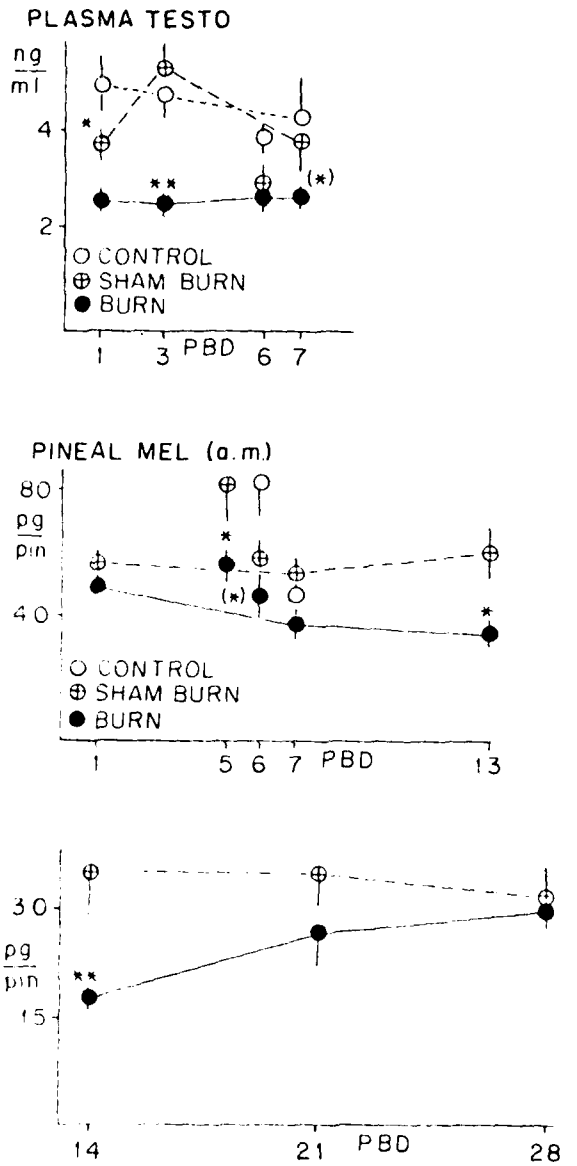

Figure 7. Plasma testosterone (Testo) and pineal melatonin (Mel) at various postburn days (PBD). PBD 6 values for testo (Experiment 4) are shown along with values from other PBD (Experiment 3). PBD 5 and 6 Mel values (experiments 6 and 4 respectively) are shown along with those from PBD 1, 7 and 13 (Experiment 5). The bottom panel represents Experiment 7. Values from the same experiment are connected with lines. *$p < 0.05$, **$p < 0.01$ burn vs sham burn. (*) $p < 0.05$ burn versus other two groups combined because of lack of significant difference between them.

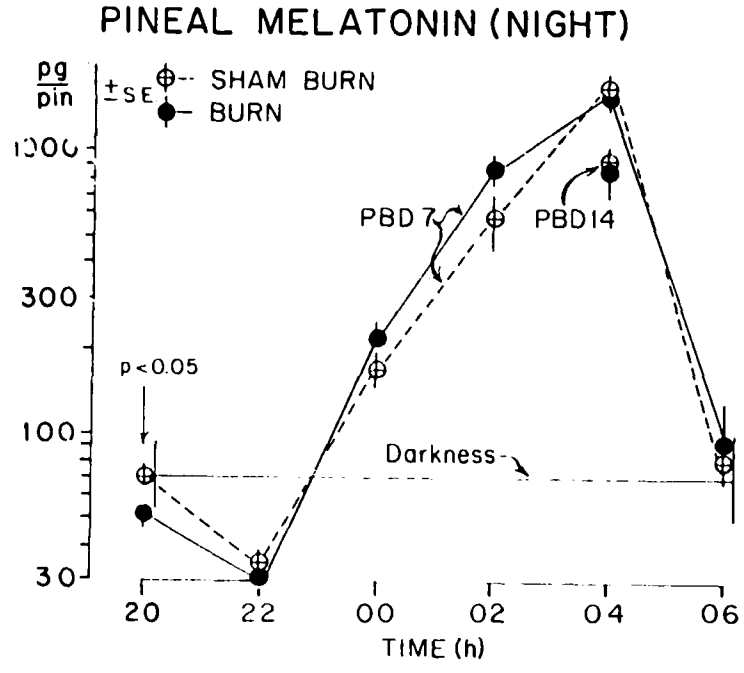

Figure 8. Nocturnal melatonin values on PBD 7 (Experiment 8) and PBD 14 (Experiment 5). Pg/pin, pg/pineal.

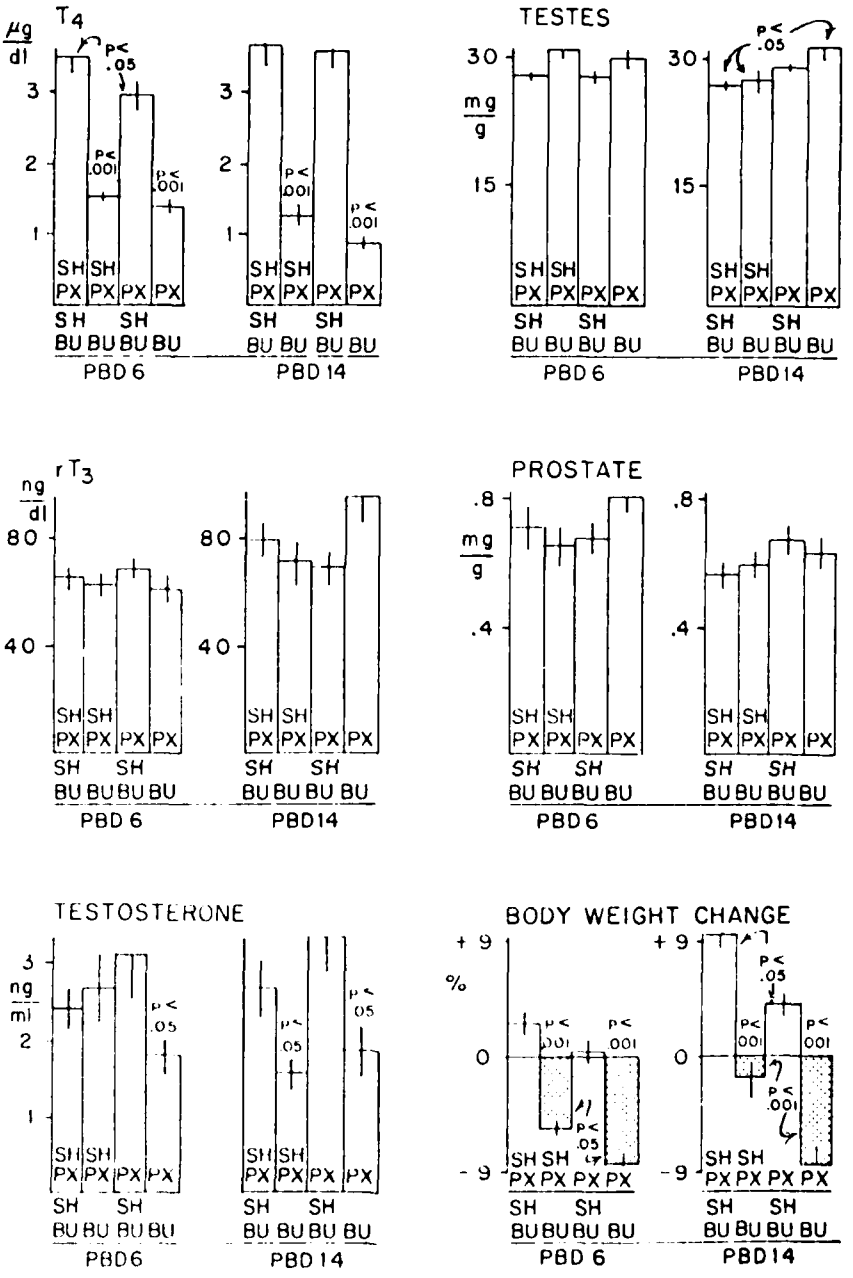

Figure 9. Plasma thyroxine (T_4), reverse T_3 (rT_3), testosterone, weights of reproductive organs relative to body weight, and body weight change relative to pre-burn weight in Experiment 9 on postburn day (PBD) 6 and 14 after sham (SH) burning (BU), BU, pinealectomy (PX) or SHPX (and the indicated combinations). Unless otherwise indicated, p values compare a bar with the one adjacent to the left.

Table IV. Reproductive organ weights for groups in Experiment 9 on postburn days (PBD) 6 and 14. Sham (SH)-pinealectomy or pinealectomy (PX) was performed two days prior to sham (SH)-burning or burning (BU).

Testes (mg)		SHPX-SHBU	SHPX-BU	PX-SHBU	PX-BU
PBD 6	Mean	3102	3267	3062	3011
	SE	56.6	98.8	72.2	127
	n	12	12	9	12
PBD 14	Mean	3226	2813*	3280	3140
	SE	85.9	124	70.1	103
	n	12	14	11	13

*$p < 0.05$ vs each remaining PBD 14 group mean.

Prostate (mg)		SHPX-SHBU	SHPX-BU	PX-SHBU	PX-BU
PBD 6	Mean	78.6	68.0	74.7	82.0
	SE	7.3	6.2	5.5	6.0
	n	12	12	9	12
PBD 14	Mean	67.4	61.0	75.7	62.7
	SE	5.6	5.6	6.1	5.5
	n	12	14	11	13

and burning cause separate and additive suppressive effects on body weight (Fig. 9). Absolute (Table IV) and relative (Fig. 9) prostate weights were unaffected by burning or pinealectomy, as were rT_3, testicular Zn and plasma Zn (Fig. 10). Liver Zn was reduced in PX-BU animals compared with that in PX-SHBU animals. Whereas the postburn reduction of plasma testosterone did not occur on PBD 6 in this experiment, it did on PBD 14 and was not prevented by pinealectomy (Fig. 9). On PBD 6, it appears that pinealectomy allowed a response to burning in plasma testosterone which was reduced in the PX BU group, implying that the intact pineal gland delays the burn-induced reduction in plasma testosterone.

DISCUSSION

Previous work from this and other laboratories led to the prediction that the pineal-induced suppression of circulating T_4 reliably observed in light-restricted hamsters was accompanied by a reduction in FT_4 concentrations, despite the associated collapse of the reproductive system and the likely resultant alteration of thyroid hormone binding proteins (1). This prediction was based partly on observed reduction of FT_4I in blinded hamsters. The present work not only confirms the pineal-dependent suppression of reproductive variables, T_4, and FT_4I after blinding, but also shows for the first time that the pineal is capable of suppressing FT_4, as measured by dialysis, in blind hamsters. Furthermore, since T_4D was unaffected and T_3U was suppressed (an expected effect of reduced total T_4), the pattern observed in the blind hamsters closely resembles that in human primary or pituitary hypothyroidism. However, the excess suppression of FT_4I compared to FT_4 indicates some similarity between the animals with activated pineals and burned patients (8). This present result could be due to pineal induction of a factor that inhibits binding of thyroid hormone to the charcoal of the T_3U test, so that the T_3U was suppressed to a greater extent than accounted for by the reduction in total T_4. Thus, several cardinal endocrine features of burned humans are produced by the pineal gland in hamsters, including reduced T_4, FT_4I and FT_4, greater suppression of FT_4I compared to FT_4, and suppression of the reproductive system. Thus, the thyroid status of burned hamsters was examined.

Burned hamsters, like burned humans, have suppressed T_4, FT_4I and FT_4 and relatively greater suppression of FT_4I than expected on the basis of FT_4 values. The suppression of FT_4 was not evident on PBD 5, but was dramatic on PBD 14, and less impressive by PBD 21. FT_4I was suppressed throughout this time period. The negative slope between FT_4I and FT_4 in PBD 14 shams (Fig. 5) is not

8. Vaughan GM, and Seraile LG: Assessment of thyroid hormone kinetics in thermally injured patients: Altered transport binding of T_4 and T_3 in burned soldiers. In U.S. Army Institute of Surgical Research Annual Research Progress Report, 1 October 1981 - 30 September 1982. U.S. Army Research and Development Command, Ft. Detrick, MD.

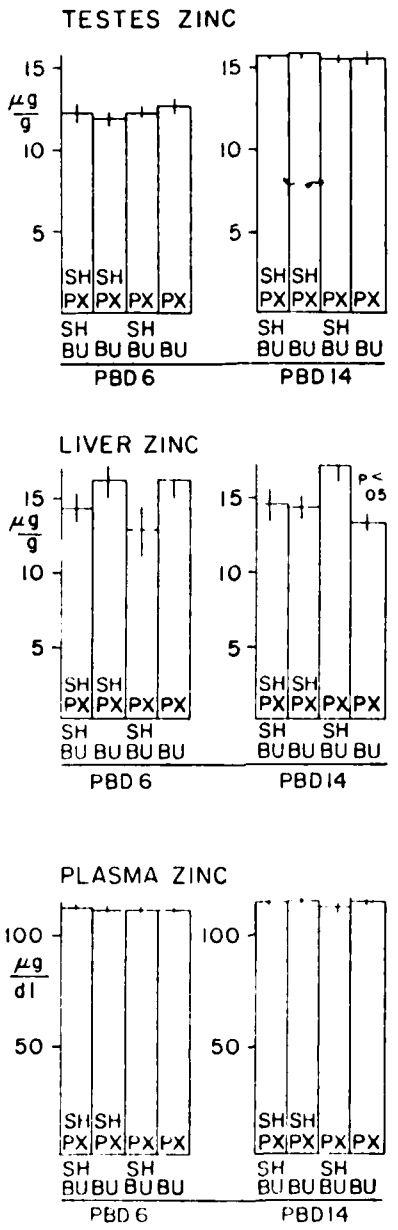

Figure 10. Tissue and plasma Zn concentrations in Experiment 9. See Figure 9 for explanation.

explained, since the slope was positive in sham burns at PBD 5 and 21. FT_4 was suppressed in burns v hether sampled during the day (0800-1000 h, Fig. 5) or at night (0400 h, Fig. 4). Compared to sham burns, T_3U was either elevated (Table II) or unchanged (Fig. 3) in burns. In other experiments (Table III) in which T_3U was the same in burns and shams, the T_4D was elevated. Thus, like burned humans (8), burned hamsters have reduced transport binding with proportionately less (if any) elevation of T_3U. Thus, the circulating factor proposed in burned humans that may inhibit binding to serum proteins (increase in T_4D) and to charcoal (less increase in T_3U, and lower FT_4I than expected for the FT_4) may also be present in the hamster model. The role of possibly reduced concentrations of thyroid hormone binding proteins remains to be elucidated.

Although one experiment showed reduction of T_3 in burns, others showed an inconsistent T_3 pattern (Fig. 3). Likewise, the reduction in rT_3 seen in some cases (Fig. 3) was absent in another (Fig. 9). Whether this inconsistency was due to the small burn size necessary in hamsters is not known. However, two variables which appear unequivocally affected by this burn in hamsters are body weight and T_4 (Fig. 6). The composite data show that the approximately 6% weight loss in burned hamsters was regained by about PBD 18. In contrast, the reduction in T_4 was much greater and had not returned to control value by PBD 21. Though the composite data indicate little effect of sham burning on body weight and T_4, individual experiments in some cases show reduction of T_4 in shams as compared to controls. Any effect of the sham procedure may have resulted from the hair clipping and, hence, reduced insulation with consequent thermoregulatory alterations.

The reduction in plasma testosterone (Fig. 7) in burned hamsters is consistent with the same finding in burned humans (Vaughan and Becker, unpublished observations).

The reduction in pineal gland melatonin content, which occurred only during the light phase (Fig. 7 and Fig. 8), was most pronounced at the end of the second week after burning, about the same time as the greatest observed reduction in FT_4 (Fig. 5). The lower melatonin values for shams and burns in one experiment (Fig. 7, bottom panel) could represent either the later time of sacrifice in the light phase or indeterminate variability among different experiments. The inability of the burn injury to affect the nocturnal surge in pineal melatonin could be due either to the small burn size or to a special effect on daytime pineal melatonin. Nevertheless, the changes in pineal melatonin suggest that the pineal gland has a role in the postburn neuroendocrine response.

We tested whether this role of the pineal might be as mediator for other neuroendocrine responses by pinealectomizing groups two days prior to the burn. The response of T_4 occurred with or without the

pineal gland. Though this could mean that the pineal may not be necessary for the T_4 response to burning, it is still possible that normal pineal activity in the weeks or months prior to a burn was necessary for the response. We must allow for this possibility, because in another species (the ferret) a delayed effect of pinealectomy on the reproductive system has been observed (9). Pinealectomy weeks or months prior to burning may be necessary to answer this question.

However, the reduction in testicular mass due to burning was prevented by pinealectomy. This indicates that at least some of the effect of burns on the reproductive system is pineal-mediated.

Because alterations in plasma and tissue Zn levels were seen after pinealectomy in rats (10), we assessed Zn concentrations in plasma, testes and perfused liver (Fig. 10). No changes due to pinealectomy or burning were observed except for a lower hepatic Zn in PX-BU than in PX-SHBU hamsters at PBD 14. The significance of this finding is obscure, except that pinealectomy may allow burning to suppress accumulation of Zn in the liver.

The most consistent endocrine findings in burned hamsters were suppression of circulating T_4 and of daytime pineal melatonin content; and, if plasma testosterone was not suppressed in the first week (as it often was), it was by the end of the second week postburn. The maximum suppression of melatonin coincided (PBD 14) with maximum suppression of FT_4 and suppression of plasma testosterone. By PBD 14, testicular weight was suppressed, and this was pineal-dependent. It may be that the postburn reduction in testicular weight would be greater by three or four weeks postburn, but it might be expected that very soon the effect would be lost as the burn heals. In contrast, the testicular weight reduction in another hamster paradigm (blinding), also pineal dependent, is much more dramatic but requires 8 to 10 weeks.

The responses in testicular weight (probably more FSH dependent) and plasma testosterone (probably more LH dependent) appeared to be dissociated with opposite effects of the pineal gland. Whereas the postburn reduction in testicular weight on PBD 14 was pineal-dependent, the reduction in testosterone was not. In fact, on PBD 6, the pineal appeared to prevent reduction in testosterone, an effect lost or overcome by PBD 14. Whether the dissociation between testicular weight and testosterone results from different gonadotrophic neuroendocrine control mechanisms affected differently by the pineal or from a separately induced accelerated testosterone disposal due to burning partially ameliorated by the intact pineal is not known.

9. Herbert J: The role of the pineal gland in the control by light of the reproductive cycle of the ferret. In Wolstenholme GEW, and Knight J (Eds.) The Pineal Gland. London: Churchill Livingstone, 1971, pp 303-329.

10. Cunnane SC, Horrobin DF, Manku MS, and Oka M: Alteration of tissue zinc distribution and biochemical analysis of serum following pinealectomy in the rat. Endocr Res Com 6:311-319, 1980.

The endocrine responses (T_4, FT_4, testosterone) to burning in hamsters resemble those in humans and, thus, the hamster model may be useful for further investigation. Furthermore, since the pineal gland is involved in the neuroendocrine response to burning in the hamster model, we will investigate pineal involvement in the response in humans. Plasma melatonin concentrations may help in this regard.

PUBLICATIONS/PRESENTATIONS

None.

Figure 1. Testicular and prostate weights in Experiment 1, as absolute (g)or relative (mg/g body weight) values 11 weeks after sham pinealectomy (SHPX), blinding (BL) and/or pinealectomy (PX).

Figure 2. Thyroxine (T_4), free T_4 index (FT_4I) and free T_4 (FT_4) in the hamsters of Experiment 1. The error lines in the upper panel (as in all other graphs) are SE. The regression line (bottom panel) is that for only the BL-SHPX group. The dotted line connects the mean point of the BL-SHPX group with the combined mean of the other two groups and provides the index of comparison for the position test using analysis of covariance (ANOCOVA). For the multiple regression, group (GRP) for each value was assigned a value of +1 or -1 as indicated.

Figure 3. Values for thyroxine (T_4), its free index (FT_4I), triiodothyronine (T_3), reverse T_3 (rT_3) and T_3 uptake (T_3U) in experiments 2 (left panel), 3 (middle panel) and 4 (right panel). Weight change indicates change from pre-burn weight. A p value over a bar compares that bar with the one adjacent to it. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$, comparing burn with sham burn or sham burn with control groups at the postburn day (PBD) indicated.

Figure 4. Thyroxine (T_4) and free T_4 (FT_4) on postburn day (PBD) 7 (early light phase) or 14 (dark phase). p values compare a bar with the adjacent one to the left.

Figure 5. Thyroxine (T_4), its free index (FT_4I) and free concentration (FT_4) in experiments 6 (left panel) and 7 (middle and right panels) on postburn days (PBD) indicated. The regression lines are based on the multiple regression analyses shown above the abscissae, except in the middle panel in which they are based on the individual group linear regressions. ANOCOVA, analysis of covariance.

Figure 6. Body weight change and circulating T_4 concentrations at various postburn days (PBD) in hamsters with total burn size (TBS) of 23% body surface. The burn weight curve results from the two regressions with their junction point rounded by hand. For the T_4, C.I.M. indicates the 95% confidence interval of the mean, and the shaded area visually approximates the range of values for the burn groups. In both graphs, each data point is a group mean.

Figure 7. Plasma testosterone (Testo) and pineal melatonin (Mel) at various postburn days (PBD). PBD 6 values for testo (Experiment 4) are shown along with values from other PBD (Experiment 3). PBD 5 and 6 Mel values (experiments 6 and 4 respectively) are shown along with those from PBD 1, 7 and 13 (Experiment 5). The bottom panel represents Experiment 7. Values from the same experiment are connected with lines. $*p < 0.05$, $**p < 0.01$ burn vs sham burn. (*) $p < 0.05$ burn versus other two groups combined because of lack of significant difference between them.

Figure 8. Nocturnal melatonin values on PBD 7 (Experiment 8) and PBD 14 (Experiment 5). Pg/pin, pg/pineal.

Figure 9. Plasma thyroxine (T_4), reverse T_3 (rT_3), testosterone, weights of reproductive organs relative to body weight, and body weight change relative to pre-burn weight in Experiment 9 on postburn day (PBD) 6 and 14 after sham (SH) burning (BU), BU, pinealectomy (PX) or SHPX (and the indicated combinations). Unless otherwise indicated, p values compare a bar with the one adjacent to the left.

Figure 10. Tissue and plasma Zn concentrations in Experiment 9. See Figure 9 for explanation.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: STUDIES OF NEUROENDOCRINE ABNORMALITIES IN BURN INJURY - INAPPROPRIATE VASOPRESSIN SECRETION (SIADH) IN BURN PATIENTS

US ARMY INSTITUTE OF SURGICAL RESEARCH
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1 October 1981 - 30 September 1982

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Unclassified

ABSTRACT

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REPORT TITLE: STUDIES OF NEUROENDOCRINE ABNORMALITIES IN BURN INJURY - INAPPROPRIATE VASOPRESSIN SECRETION (SIADH) IN BURN PATIENTS

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To determine if concentration of plasma arginine vasopressin (AVP) is inappropriate for the plasma Na^+ concentration in hyponatremic burn patients, we obtained 32 plasma samples from 20 patients with total burn size (TBS) 15 to 80% of body surface on or after postburn day (PBD) 4 in the morning following all-night recumbency. In the 25 samples (17 patients) with hyponatremia, AVP was elevated, 1.6 to 14.3 (normal < 0.5) pg/ml. Most patients with normal serum Na^+ had normal AVP values. Out of the total, nine patients (12 samples) without renal failure or sepsis, selected also for hyponatremia and urinary $Na^+ \geq 20$ mEq/L, were considered separately. BUN of 11.7 ± 1.18 mg/dl and plasma glucose of 130 ± 5.6 mg/dl, Na^+ of 130 ± 1.1 mEq/L, calculated osmolality of 272 ± 1.6 mosm/kg, and cortisol of 20.4 ± 1.6 µg/dl were associated with a 24-hour fluid intake of 4.3 ± 0.26 L and urinary output of 2.7 ± 0.33 L, Na^+ of 80 ± 14 mEq/L, and osmolality of 520 ± 73 mosm/kg (mean $\pm$ SE). In all of the plasma samples, AVP was markedly elevated (6.9 ± 1.1 pg/ml). In another study, five hyponatremic burn patients were given a standard water load. Excretion of the water was delayed, and further dilution of the initially hypotonic plasma resulted in a fall of urinary osmolality and plasma AVP. Cutaneous thermal injury can cause resetting of the mechanism linking plasma tonicity and AVP secretion resulting in dilutional hyponatremia. This syndrome occurs in the absence of gross physiological perturbations such as volume depletion or adrenal insufficiency.

Plasma arginine vasopressin
Hyponatremia
Urinary osmolality

INAPPROPRIATE VASOPRESSIN SECRETION (SIADH) IN BURN PATIENTS

Antidiuresis in the first 24 to 36 hours following trauma has been observed for many years and was reviewed by Dudley et al (1). Using major surgery as a model, these authors also found marked water retention in the first one to two days after surgery that could be mimicked by administration of exogenous posterior pituitary extract. They proposed that post-traumatic antidiuresis was not entirely explained by sodium retention but resulted from secretion of antidiuretic hormone (ADH) and suggested that confirmation of this mechanism awaited an assay for ADH.

Soroff et al. (2) found that exaggerated antidiuresis often exists for days and weeks after burn injury. They observed that in burn patients exhibiting a fall in serum Na^+ concentration, there was an associated administration of greater amounts of electrolyte-free water than in other burn patients. Adequate urine flow (mean 2.7 L/day), appreciable Na^+ excretion (64 mEq/L), and positive Na^+ balance indicated that a deficit of fluid volume or of Na^+, a possible cause for water retention, was not a factor in these cases. Instead, they suggested that hyponatremia in the presence of burn injury is dilutional and speculated that it results from an osmoregulatory mechanism set a lower than normal plasma tonicity.

Following this, the clinical syndrome of inappropriate secretion of antidiuretic hormone (SIADH) was described in patients with cancer, disorders of the central nervous system, and diseases of the lung, and the clinical criteria for the diagnosis of SIADH were defined (3). Collentine et al. (4) reported three burn patients exhibiting the criteria of hyponatremia and hypotonic plasma, urine not maximally dilute, and normal renal and adrenal function. They suggested that burn injury could cause SIADH.

Subsequent development of a radioimmunoassay for plasma ADH (arginine vasopressin, AVP) allowed confirmation of elevated plasma AVP as the mechanism of classical SIADH (5). Application of AVP

1. Dudley HF, Boling EA, LeQuesne LP, and Moore FD: Studies on antidiuresis in surgery: Effects of anesthesia, surgery and posterior pituitary antidiuretic hormone on water metabolism in man. Ann Surg 140:354-367, 1954.

2. Soroff HS, Pearson E, Reiss E, Artz CP: The relationship between plasma sodium concentration and the state of hydration of burned patients. Surg Gyn Obstet 102:472-482, 1956.

3. Bartter FC, and Schwartz WB: The syndrome of inappropriate secretion of antidiuretic hormone. Am J Med 42:790-806, 1967.

4. Collentine GE, Waisbren BA, and Lang GE: Inappropriate secretion of antidiuretic hormone as an accompaniment of burn injury. In Matter P, Barclay TL, and Konickova A (Eds). Research in Burns. Transactions of the Third International Congress on Research in Burns. Bern, Switzerland: Hans Huber Publishers, 1971.

5. Robertson GL, Shelton RL, and Athar S: The osmoregulation of vasopressin. Kidney Internat 10:25-37, 1976.

assays to plasma of burn victims (6,7) has been limited to the first postburn week and has demonstrated very high concentrations of AVP in the presence of high plasma tonicity. Initially high plasma osmolality may have resulted from the fluid shifts that occur just after injury and during the first few days when fluid resuscitation is the prime goal of therapy. However, by postburn day 4 to 6, one can see in those data a suggestion of low plasma tonicity at a time when plasma AVP was still elevated. In those studies, serum Na^+ concentrations were not presented, and in one (6), it is stated that serum Na^+ stayed within normal limits. Thus, the possibility of SIADH was not evaluated in those studies.

We have focused our attention on burn patients with hyponatremia occurring after the third postburn day when circulating volume has been restored (8). Measurements of plasma AVP corroborate the presence of SIADH in these patients.

MATERIALS AND METHODS

Patients. Men, aged 17 to 63 years, were studied on postburn day (PBD) 4 to 58 with initial total burn size (TBS) of 15 to 80% of the body surface area. Prior to their accidental burn injury, they had no history of previous endocrine or renal disease. They were resuscitated according to a modified Brooke Formula (9) in the first 48 hours after injury. Subsequently, fluids and electrolytes were administered to replace losses in a manner guided partly by urine flow and determinations of body weight, electrolytes, urea nitrogen, and creatinine in serum and urine. A large caloric intake (estimated resting metabolic rate + 25%) was begun in the first week. Morphine was given if required for pain. Wounds were treated with alternate application of mafenide acetate in the morning and silver sulfadiazine in the evening. When eschar excision and grafting occurred prior to our studies, at least five days elapsed before the patient was studied. Samples were taken after overnight recumbency and before breakfast or other elements of routine care were given.

6. Hauben DJ, LeRoith D, Glick SM, and Mahler D: Nonoliguric vasopressin oversecretion in severely burned patients. Israel J Med Sci 16:101-105, 1980.

7. Morgan RJ, Martyn JAJ, Philbin DM, Coggins CH, and Burke JF: Water metabolism and antidiuretic hormone (ADH) response following thermal injury. J Trauma 20:468-472, 1980.

8. Pruitt BA Jr, Mason AD Jr, and Moncrief JA: Hemodynamic changes in the early postburn patient: the influence of fluid administration and of vasodilator (hydralazine). J Trauma 11:36-46, 1971.

9. Pruitt BA: Fluid resuscitation for extensively burned patients. J Trauma 21:690-692, 1981.

Analyses. Electrolytes, urea nitrogen, glucose and creatinine were determined in serum and urine by standard procedures. Plasma cortisol was determined by radioimmunoassay. Osmolality was determined by freezing point depression. Plasma arginine vasopressin (AVP; antidiuretic hormone) was determined by radioimmunoassay (10).

Study I (Figs. 1-3). Blood was sampled from 20 burn patients for determination of electrolytes and AVP in plasma. Patients were included if they were in the intensive care area or if they were known to have been hyponatremic. Some patients were sampled on two separate mornings for a total of 32 samples. Urine samples were also obtained on some of these occasions for determination of Na^+ and osmolality. The relationship between plasma Na^+ and AVP was compared to that in a large group of uninjured normal subjects (Fig 1). In order to eliminate volume deficit and other factors as explanations for possibly elevated AVP values, 12 samples from 9 patients (TBS 15 to 48%, PBD 4 to 21) with hyponatremia, serum creatinine $\leq$ 1.3 mg/dl, urinary $Na^+ \geq$ 20 mEq/L, normal blood pressure and chest radiographs, and absence of clinical evidence of sepsis (ileus or obtundation) were considered separately (Figs. 2 and 3). Cortisol was determined in the plasma samples. Because plasma osmolality was not measured in these patients, it was calculated from the concentrations of the significant osmotically active plasma components ($2Na^+$ + BUN/2.8 + glucose/18). In addition, fluid intake and urinary output were determined for the 24 hours immediately preceding the time of plasma sampling for the study.

Study II (Figs. 4-6 and Table I). Five other patients with hyponatremia, normal BUN and serum creatinine were given a water load orally, 20 ml/kg body weight, over 20 minutes. Na^+ concentration and osmolality were measured in plasma and in hour-long urine collections taken prior to and for 4 to 6 hours after the beginning of water ingestion. AVP concentration was determined in plasma at baseline and at hourly (patients 3-5) or two-hourly (patients 1,2) intervals. Patient 1 had H. influenzae epiglottitis and pneumonia at the time of study and was on a respirator with inspiratory assistance and a positive end-expiratory pressure of 3 cm of water. The other four patients had no pulmonary disease or sepsis at the time of study. Patient 4 had a Swan-Ganz catheter placed the day before for determination of pulmonary wedge pressure and cardiac output by thermodilution. In this patient, 4 hours after ingestion of the water load, an infusion of 5% NaCl, 0.05 ml/kg per minute, was given for two hours, and plasma Na^+ and osmolality were determined at 20-minute intervals. Plasma cortisol was determined in patients 1-4 from samples taken in the morning within 1-3 days of the study.

10. Robertson GL, Shelton RL, and Athar S: The osmoregulation of vasopressin. Kidney Internat. 10:25-37, 1976.

Table I. Characteristics and baseline values for the patients receiving the water loading test.

Patient	Age (Yr)	TBS (%)	PBD	Blood Pressure (mmHg) Systolic	Diastolic	Pulse (min^{-1})	Temperature (rectal, ^{o}F)	Na^{+} (mEq/L) Plasma	Urine
1	58	21	11	138	70	130	101.0	127	96
2	25	17	9	148	79	110	99.2	133	19
3	63	29	37	153	76	107	99.6	129	105
4	25	42	14	150	70	134	100.8	132	10
5	59	32	113	144	88	136	100.8	132	138

TBS, total burn size as % of body surface area. PBD, postburn day.

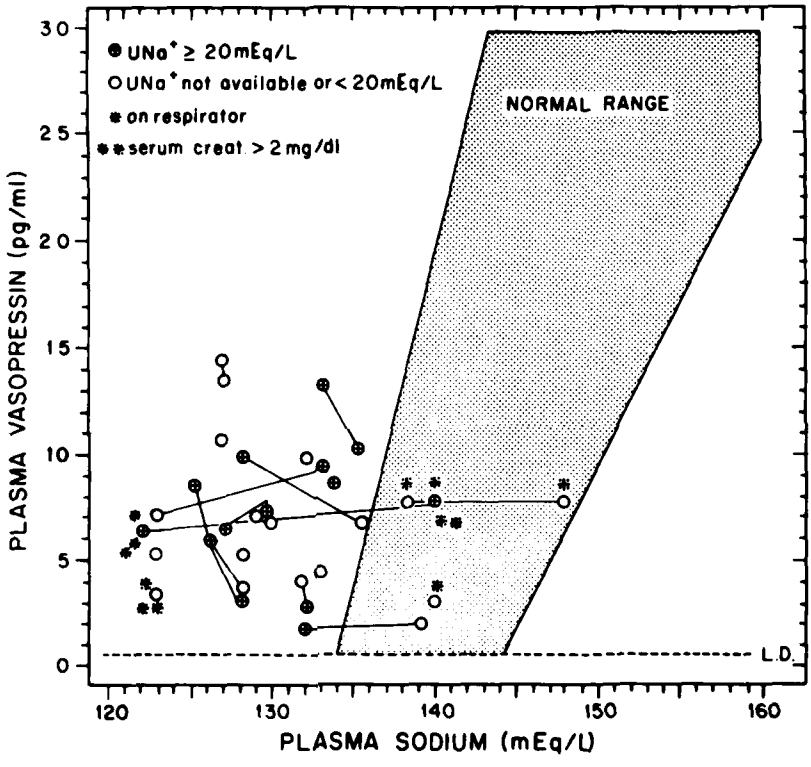

Fig. 1. Plasma Na^+ and AVP concentrations in burn patients. The lines connecting two symbols indicate the same patient sampled on two different mornings. The stippled area represents the normal range (G.L. Robertson, unpublished). UNa^+, urinary Na^+ concentration. Creat., creatinine concentration. L.D., least detectable AVP level. The established range for normal subjects is shown.

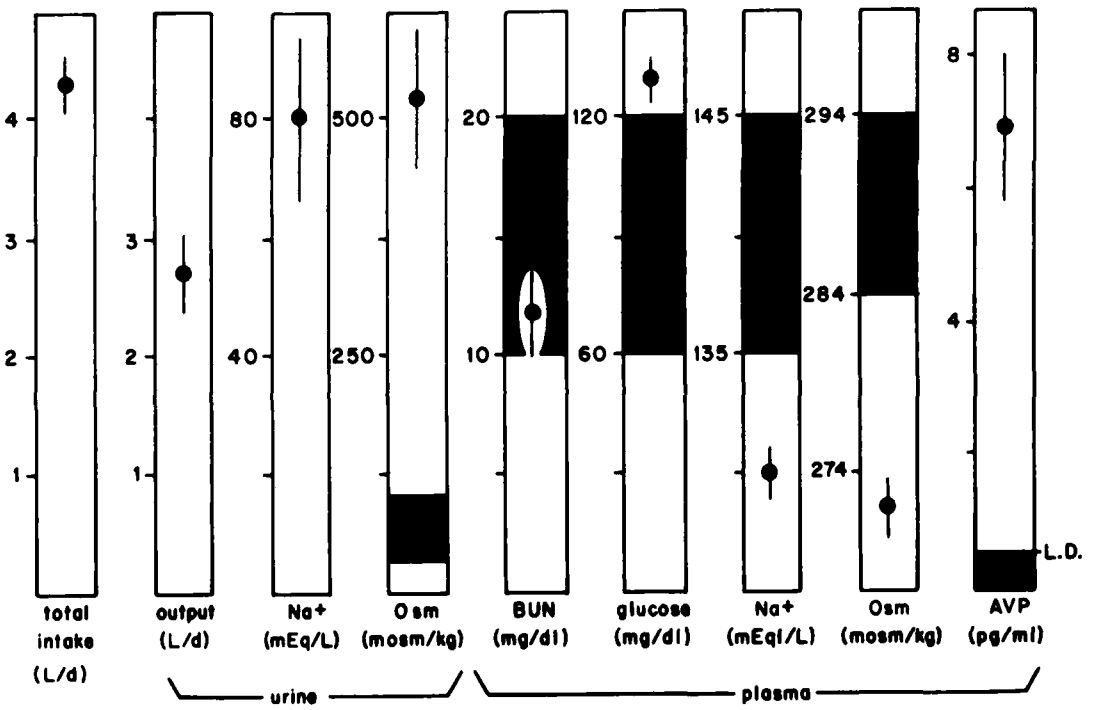

Fig. 2 Urinary and plasma (mean ± SE) values in 9 patients with normal renal function, hyponatremia and urinary Na^+ concentration ≥ 20 mEq/L. Blocked areas indicate the normal ranges. For urinary osmolality (Osm) and plasma AVP, the normal range for hypotonic plasma is given.

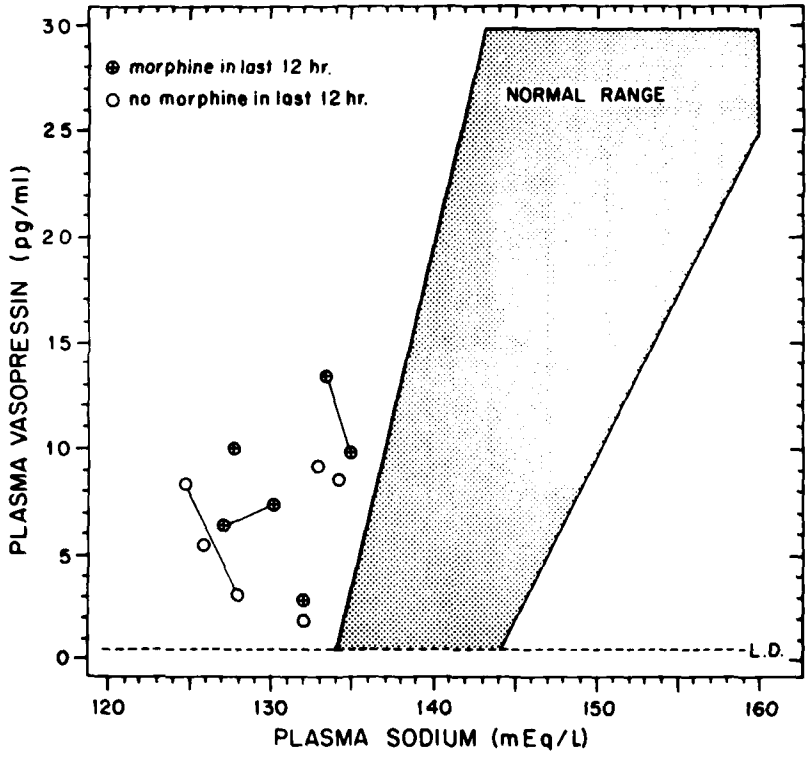

Fig. 3. Comparison of plasma Na^+ and AVP concentrations for the individual patients of Fig. 2.

FIGURE 4.

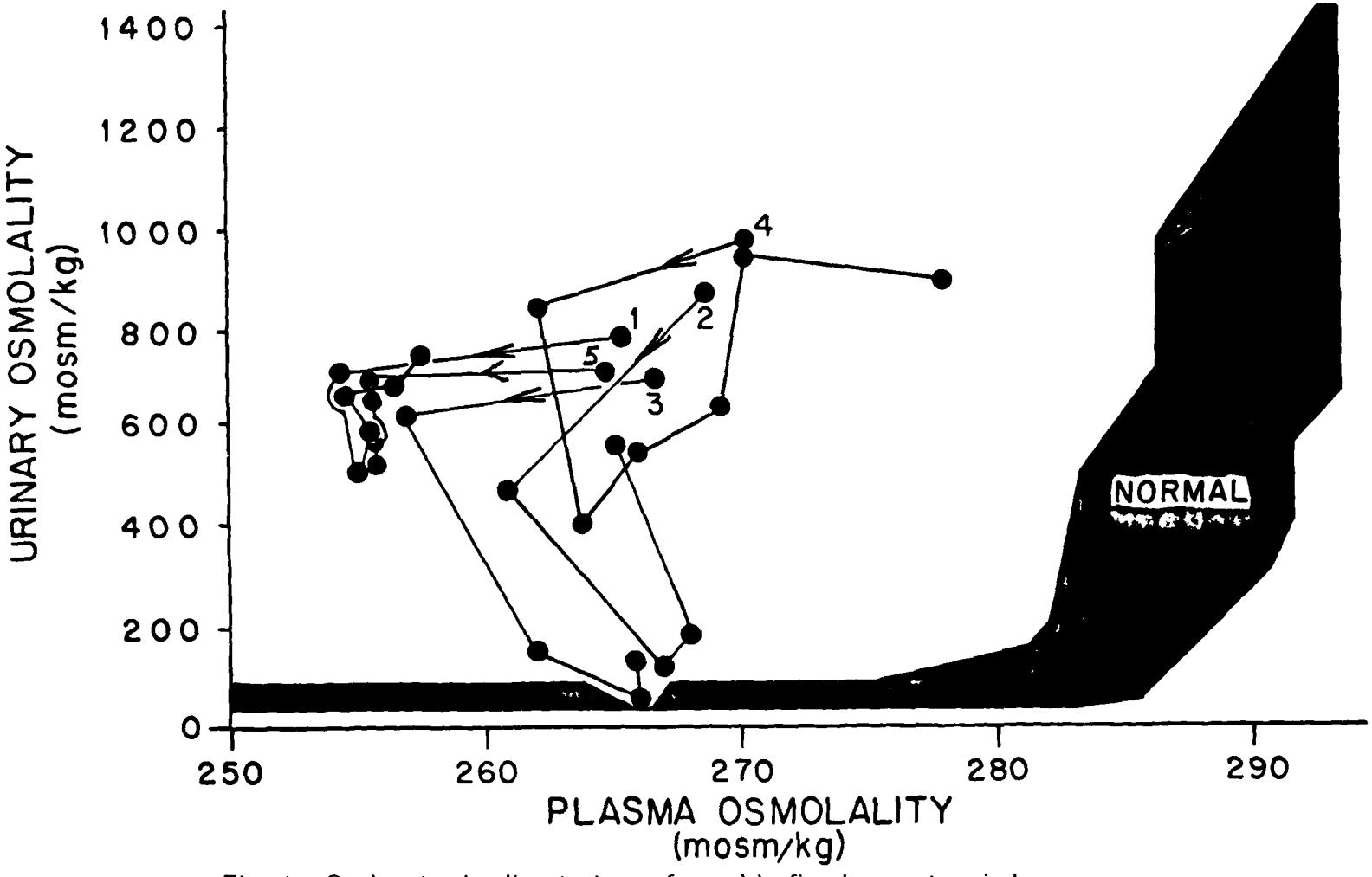

Fig. 4. Oral water loading tests performed in five hyponatremic burn patients. The baseline sample is indicated by the patient number, and subsequent samples are 1 hour apart. The last two samples of patient 4 were obtained at 1- and 2-h during an infusion of 5% NaCl. The established range for normal subjects is shown.

FIGURE 5.

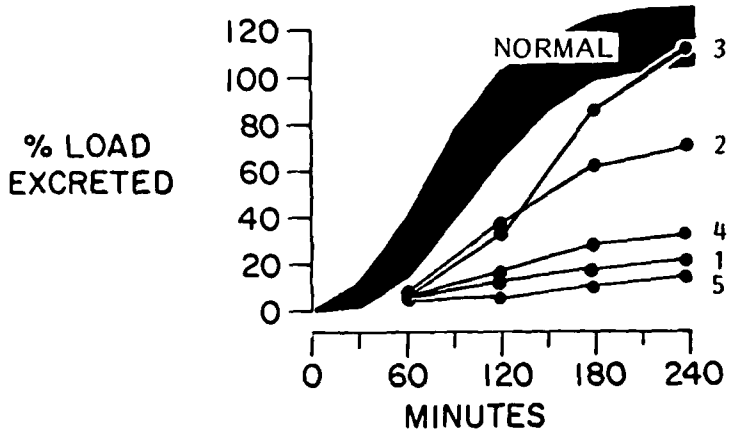

Fig. 5. Cumulative urinary volume excreted during the water loading tests. Patient numbers are indicated at the right. The established range for normal subjects is shown.

FIGURE 6.

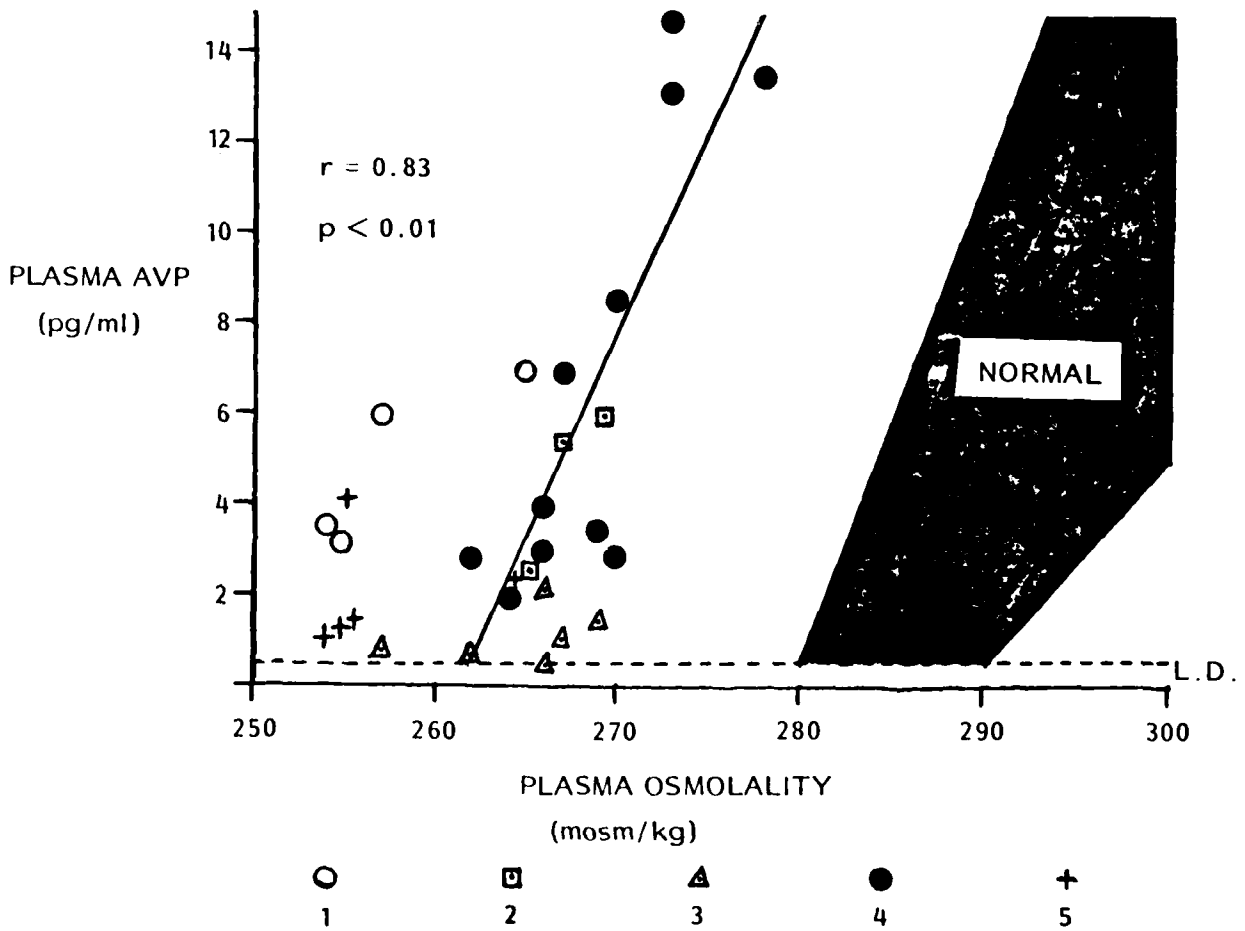

Fig. 6. Comparison of plasma osmolality and AVP during the water loading tests. Patient 4 was also sampled every 20 min during an infusion of 5% NaCl immediately following the 4-hour water loading test (his 6 highest AVP values indicate samples taken during the 5% NaCl infusion). L.D., least detectable AVP level. The established normal range is indicated. The key below the abscissa indicates patient numbers. The indicated linear regression is for patient 4.

RESULTS

Study I. In 27 plasma samples, AVP was elevated beyond that anticipated for the plasma Na^+ concentration in uninjured normal subjects (Fig. 1). In 25 samples (17 patients), hyponatremia (Na^+ 123 to 134 mEq/L) was present and AVP ranged from 1.6 to 14.3 (normal < 0.5) pg/ml. Only two of these patients, both of whom were on respirators and one of whom was hypotensive and receiving a dopamine infusion, had elevated serum creatinine. Of 7 samples (6 patients) with plasma Na^+ 135 to 148 mEq/L, 5 samples (4 patients) had AVP values in the normal range for the plasma Na^+. Three of these patients with normal AVP were on respirators, one of these had an elevated serum creatinine, and another was hypotensive and receiving dopamine.

In the group of nine uncomplicated patients (Figs. 2, 3) selected for hyponatremia and urinary $Na^+ \geq 20$ mEq/L, concentrations of plasma cortisol (range 13 to 32 µg/dl) were in the normal range (7 to 25 µg/dl) or elevated. Despite appreciable urine production and Na^+ excretion, the low plasma Na^+ concentration and calculated plasma osmolality were associated with a high measured urine osmolality (Fig. 2). Fig. 3 shows that in each sample, plasma AVP was elevated (> 0.05 pg/ml) for the Na^+ concentration, whether or not morphine was given for pain in the preceding 12 hours. Morphine had not been given within 24 hours prior to collection of four of the samples (in three patients).

Study II. Just prior to the water loading test, these patients exhibited diluted plasma, concentrated urine (Fig. 4), hyponatremia and detectable urinary Na^+ (Table I). Patient 4, who had a Swan-Ganz catheter and in whom a 2-hour infusion of 5% NaCl followed the water loading test, had a pulmonary wedge pressure of 13 mm Hg at baseline and 17 mm Hg 6 hours later at the end of the NaCl infusion. Cardiac output, obtained only at baseline, was 17.2 L/minute in this patient. Morning plasma cortisol (obtained in patients 1-4) ranged 9.6 to 26.8 µg/dl. Fig. 4 shows that after the water load, further reduction in measured plasma osmolality was followed by a reduction in urinary osmolality in every case. However, relative concentration of urine with respect to the plasma, together with delayed excretion of the water load (Fig. 5), indicates the propensity for water retention in these patients. Patient 3, who responded with the greatest urinary dilution (though at an abnormally low plasma tonicity) also finally excreted the water load by 4 hours.

AVP values (Fig. 6) from these patients confirm the observation from Study I that plasma AVP concentration is inappropriately elevated for the plasma tonicity in hyponatremic burn patients. Reduction of plasma osmolality was accompanied by a fall in AVP concentration. For patient 4, in whom the addition of the hypertonic saline infusion allowed more samples and a greater range of plasma tonicity, plasma AVP was significantly correlated with plasma tonicity and a leftward displacement of the relationship was evident with respect to normal.

DISCUSSION

Observation of hyponatremia and hypotonic plasma in burn patients in the presence of hypertonic urine confirms the case reports of Collentine et al. (4) and indicates that classical SIADH can occur in burn patients. Furthermore, elevated AVP concentrations have been observed in these patients and can be lowered by further dilution of plasma, with a fall in urine concentration. These results indicate in patients with burn injury, that the SIADH is the result of measurably elevated plasma concentrations of AVP and that the threshold for AVP secretion is set at a lower than normal plasma tonicity. Normal or elevated plasma cortisol concentrations indicated absence of adrenocortical failure, a potential cause for water retention and SIADH (11).

Plasma concentrations of norepinephrine and epinephrine are markedly elevated in burn patients (12). However, it is unclear what net effect this might have on AVP secretion, because infusion of norepinephrine inhibits, whereas infusion of isoproterenol stimulates water retention in dogs, apparently through alterations in AVP secretion (13). Hypothyroidism is associated with elevated plasma AVP (14). Though burn patients typically have low plasma concentrations of total and free triiodothyronine, the metabolic significance of this is not known, because burn patients are hypermetabolic (12).

Angiotensin II, particularly in vitro with posterior pituitary tissue or when given by the intracerebroventricular route, can promote the

11. Linas SL, Berl T, Robertson GL, Aisenbrey GA, Schrier RW, and Anderson RJ: Role of vasopressin in the impaired water excretion of glucocorticoid deficiency. Kidney Internat 18:58-67, 1980.

12. Vaughan GM, and Becker RA: Thyroid hormones and catecholamines in patients with severe burns: A hypermetabolic low T_3 syndrome. In Ziegler MG, and Lake CR (Eds). Norepinephrine. Baltimore: Williams and Wilkins Company, 1982, in press.

13. Schrier RW, Berl T, Anterson RJ, and Mcdonald KM: Nonosmolar control of renal water excretion. In Antreoli TE, Grantham JJ, Rector FC Jr (Eds). Disturbances in Body Fluid Osmolality. Bethesda, Maryland: Am Physiol Soc, 1977, pp 149-178.

14. Skowsky WR, and Kikuchi TA: The role of vasopressin in the impaired water excretion of myxedema. Am J Med 64:613-621.

secretion of AVP in animals (13,15,16). Plasma renin activity (despite normal plasma volume and Na^+ excretion) (17) and angiotensin II concentrations in plasma (18) are reportedly elevated in burn patients. Thus, there is some liklelihood that elevated plasma angiotensin II, possibly resulting from elevated sympathetic activity or from as yet unidentified stimuli, may be a factor in SIADH of burn injury.

Although morphine or opioids have been shown to inhibit (15,16,19, 20 21) or promote (22,23) AVP secretion, morphine administration did not appear to be a necessary factor in the elevated plasma AVP concentrations in burn patients. Pain is also an unlikely factor, because those not requiring morphine denied being in pain.

Because blood flow to the burn wound is increased (24), one might consider whether this shunt results in a decreased flow to non-injured

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areas and consequently a decrease in effective arterial volume which could stimulate AVP release. However, the available evidence suggests that areas outside the injury do not have compromised flow. Muscle blood flow was 7% higher (not statistically significant) in burn patients compared to controls (25). In burn patients, blood flow in an uninjured leg was 14% higher (not statistically significant) than in control subjects (24). The kidney may be of particular interest, because an experimental arteriovenous shunt may produce a reduction in renal plasma flow and creatinine clearance (26). But, blood flow in the splanchnic bed of burn patients was markedly elevated to twice normal values, and renal blood flow in burn patients was normal with Na^+ excretion <40 mEq/day and elevated with greater Na^+ excretion (27). Glomerular filtration rate is reportedly elevated in burn patients (28). Because of increased gluconeogenesis, burn patients have elevated urea production (29). Thus, the BUN values in the lower normal range and appreciable urine volumes in our patients further suggest adequate effective volume. Whether the hypermetabolic state and increased O_2 demand (29) influence the adequacy of the effective arterial flow with respect to AVP control mechanisms is not known, and this may represent one of the difficulties in assessing the role of effective arterial flow in the SIADH of burn injury. Tachycardia, a feature of the hemodynamic status after burn injury, by itself has a diuretic effect, apparently through reduction of AVP secretion (13). Since this is a response opposite to the antidiuresis seen in burn patients, tachycardia does not explain the SIADH in these patients.

Thus, the SIADH in burn patients seems not to be a result of gross physiological perturbations, such as adrenal failure or volume contraction. Several potential mechanisms may be fruitful areas for future investigation, including the net effect of elevated plasma catecholamines and angiotensin II and reduced thyroid hormone concentrations. In addition, the possibility of reduced effective arterial volume relative to the increased metabolic needs requires further investigation before it can be excluded as a contributory mechanism. Regardless of the mechanism, urine concentration in burn patients can be inappropriate, as judged by the plasma

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tonicity, but does fall with further dilution of plasma. This suggests a resetting of the osmostat for control of plasma tonicity, with a lower plasma tonicity threshold at which AVP is released. Studies over a wider range of plasma tonicity in a larger number of patients will be needed to determine if there is an additional altered sensitivity of AVP release to increments in plasma tonicity above threshold. The observed altered control of plasma tonicity is not surprising in view of other previously observed burn-injury-related derangements of hypothalamic function. These include increased sympathetic activity, elevated heat production and core-skin heat conductance, self selection of a warmer ambient temperature of maximal comfort despite a higher core and mean skin temperature in burn patients than in control subjects, blunted growth hormone response to provocative stimuli (29) and failure of plasma thyrotrophin to rise despite low concentrations of thyroid hormones (12).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0G 6968	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. CHANGE	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BB	303
b. CONTRIBUTING				
c. XXXXXXXXXX	STOG 80-7	2:5		

11. TITLE (Precede with Security Classification Code)*

(U) Alteration of Host Resistance in Burned Soldiers

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 10	Cont	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	5.0	180
	CURRENT 83	5.0	260

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Institute of Surgical Research

ADDRESS:* Fort Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL

NAME: Basil A. Pruitt, Jr., MD, COL, MC

TELEPHONE: 512-221-2720

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

20. PERFORMING ORGANIZATION

NAME:* US Army Institute of Surgical Research
Microbiology Branch

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME:* Albert T. McManus, Ph.D., MAJ, MSC

TELEPHONE: 512-221-3411

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME: POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Tissue Spreading Factors; (U) Rat Model; (U) Infection; (U) Immunostimulants; (U) Virulence Factors; (U) Plasmids; (U) Antibiotic Effects

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To define the microbial basis of opportunistic infection in susceptible burned soldiers, identify specific mechanisms of decreased host resistance that are targeted by opportunistic pathogens and develop and evaluate countermeasures.

24. (U) The high susceptibility of burned rats to experimental infection with Pseudomonas aeruginosa and Proteus mirabilis will be investigated. The effect of in vitro alterations of specific microbial characteristics on infection will be investigated. Specific antimicrobial and immunostimulator therapies will be examined.

25. (U) 8110 - 8209. The clinical trial of the experimental cephalosporin antibiotic Cefsulodin sodium (Abbott) was completed. A total of 10 patients were included in the study. The interpretation of the trial results is in progress. In vitro sensitivity of Pseudomonas aeruginosa to Cefsulodin remained high during the trial. A new topical antimicrobial agent is being evaluated in animals. The agent (WP-973, Westwood) is highly active in Pseudomonas aeruginosa infected rats and Proteus mirabilis infected rats. In vitro activities indicate this compound may have broader activity than presently available topical antimicrobial agents.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

REPORT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1981 - 30 September 1982

Investigators:

Albert T. McManus, Ph.D., Major, MSC
Arthur D. Mason, Jr., M.D.
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Camille L. Denton, M.A.
George T. Daye, Jr., M.A.
Virginia C. English, M.A.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

REPORT TITLE: ALTERATIONS OF HOST RESISTANCE IN BURNED SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1981 - 30 September 1982

Investigators: Albert T. McManus, Ph.D., Major, MSC
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Reports Control Symbol MEDDH-288(R1)

The clinical trial of the experimental cephalosporin antibiotic cefsulodin sodium (Abbott) was completed. A total of 10 patients were included in the study. The interpretation of the trial results is in progress. In vitro sensitivity of Pseudomonas aeruginosa to cefsulodin remained high during the trial. A new topical antimicrobial agent is being evaluated in animals. The agent (WP-973, Westwood) is highly active in Pseudomonas aeruginosa infected rats and Proteus mirabilis infected rats. In vitro studies indicate this compound may have broader activity than is available in currently used topical antimicrobial agents.

Tissue spreading factors
Rat model
Infection
Immunostimulants
Virulence factors
Plasmids
Antibiotic effects
Humans

ALTERATIONS OF HOST RESISTANCE IN BURNED SOLDIERS

EXPERIMENTAL PARENTERAL AGENTS

In vitro susceptibility to the investigational cephalosporin class antibiotic cefsulodin sodium (Abbott) was measured in 798 burn patient isolates of *Pseudomonas aeruginosa*. The organisms tested were from 68 patients. Sensitivity measurements were made by disc diffusion agar overlay technique with 30 mcg discs. Significant resistance to this antibiotic was noted during the reporting period. A comparison of *in vitro* sensitivity during the past three reporting periods is presented in Table 1. The distribution of inhibition zones for 1981 and 1982 is presented in Figure 1. As can be seen, a significant shift to the left is obvious in 1982. The decreased susceptibility to cefsulodin did not relate temporally to the clinical trial conducted with this agent. No resistant Pseudomonas was found to occur in patients treated with cefsulodin. In fact, significant resistance developed more than 5 months after completion of the trial. The selective pressure for cefsulodin resistance in the absence of clinical use of cefsulodin is unknown. The most likely mechanism appears to be a cross-resistance between cefsulodin and moxalactam, another third generation cephalosporin. Moxalactam was in use during the period of increased cefsulodin resistance, and 131 of the 143 cefsulodin resistant strains were also resistant to moxalactam (92%).

A summary of the clinical study completed during the reporting period is presented in a later section of this report.

Two other investigational antibiotics are currently being examined *in vitro*, Ceftazidime (GLAXO GR 20263), a new beta-lactamase resistant cephalosporin, and the novel antibiotic N-formimidoyl thienamycin (Merck MK 0787). Results will be detailed in future reports.

EXPERIMENTAL TOPICAL ANTIMICROBIAL AGENTS

A candidate topical antimicrobial agent WP-973 (Westwood) has been examined in experimental animals. The structure of the compound is presented in Figure 2. WP-973 was examined as a 2% w/w cream in the Institute's standard *Pseudomonas aeruginosa* strain 59-1244 and *Proteus mirabilis* 77-82234 infected burn rat models. Treatment was initiated 24 hours after burning and infecting in the Pseudomonas model and after 4 hours in the Proteus model. Trial data are presented in Table 2. WP-973 was an effective agent in both models. It should also be noted that silver-sulfadiazine was, as expected, an active agent. It appears that WP-973 is generically unrelated to silver-sulfadiazine and therefore represents a totally new class of effective topical agents.

EXPERIMENTAL VACCINES

The experimental polyvalent *Pseudomonas aeruginosa* vaccine PEV-01 (Wellcome) has been evaluated in burned rats. Male Sprague-Dawley rats, 180-200 g with 20% full-thickness wounds, were used. Animals were divided

Table 1. Cefsulodin Sodium Activity against Burn Patient *Pseudomonas aeruginosa*

	1980 (% Resistant)	1981 (% Resistant)	1982 (% Resistant)
Strains resistant / Strains sensitive	5/76 (6.0)	8/556 (1.4)	143/655 (17.9)

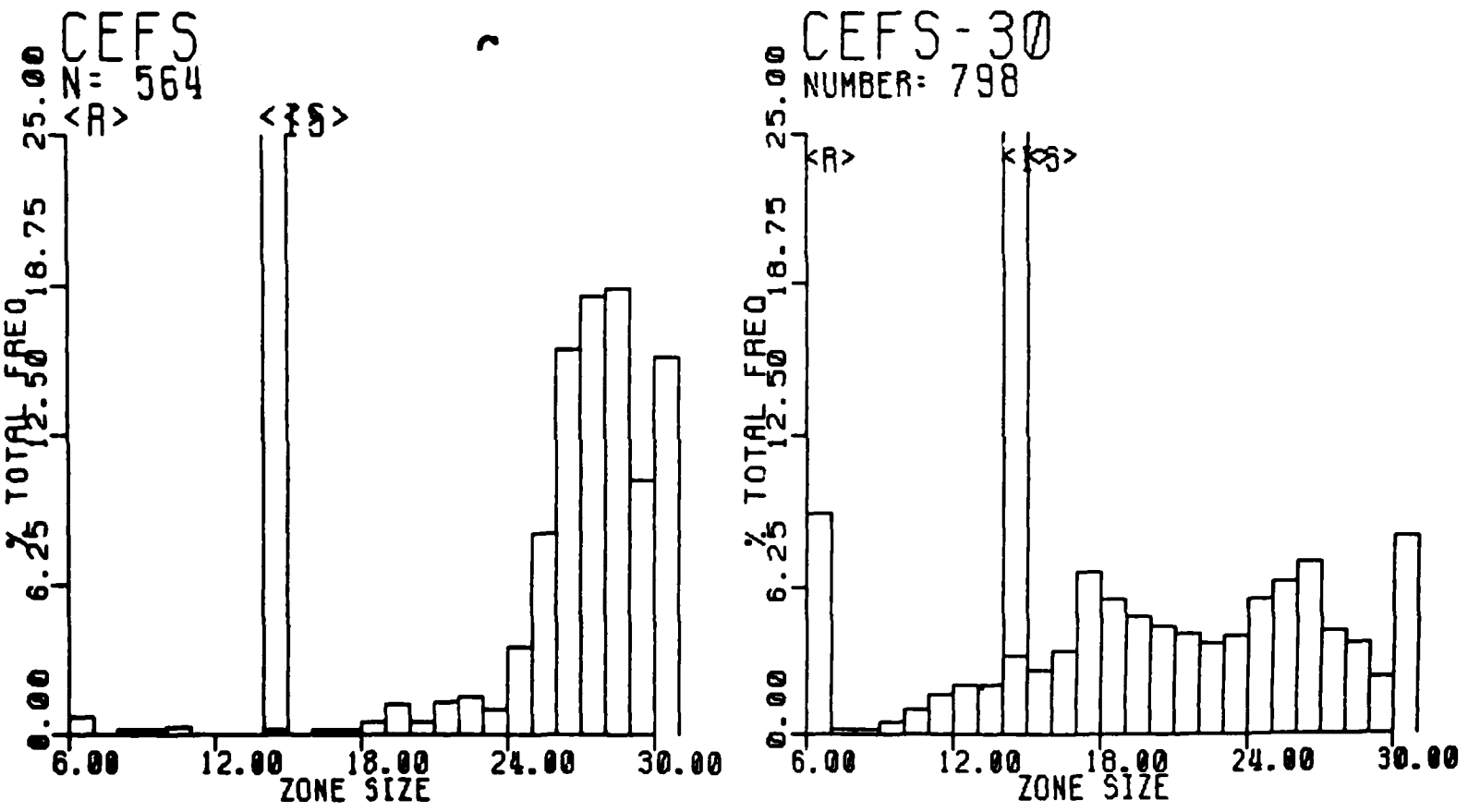
1982 vs. 1981 + 1980 $P < 0.01$ 1982 more resistant.

Table 2. Topical Chemotherapy with Chlorhexidine Diphosphanilate (WP-973)

	WP-973	Placebo	Infected	AgSD	Burn only
Pseudomonas aeruginosa strain (59-1244)	13/40*	39/41	39/39	11/34*	2/30
Proteus mirabilis (77-82234)	11/41*	35/42	35/42	8/39*	2/39

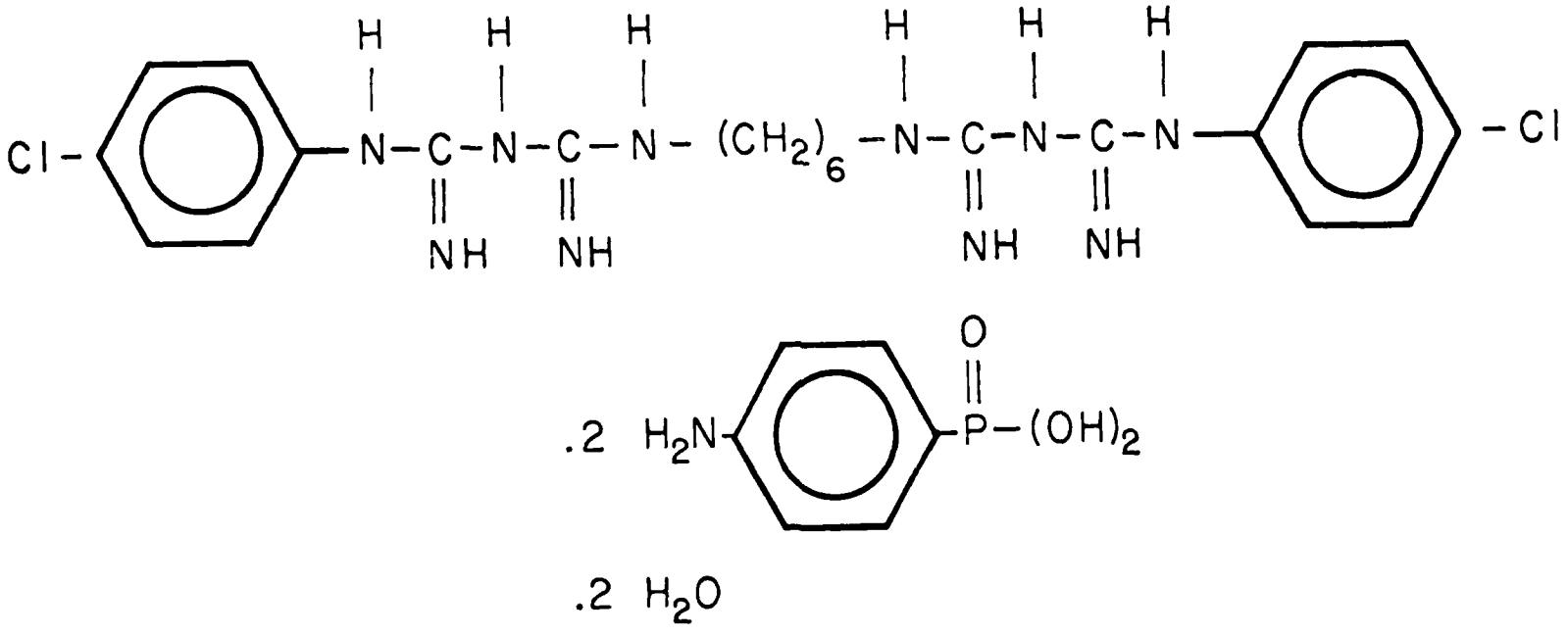
Numbers represent deaths/total tested.

* $P < 0.001$ improvement in survival over infected and placebo groups.



a b

Figure 1. Frequency distribution of zones of inhibition of Pseudomonas aeruginosa strains using 30 µg disc of cefsulodin sodium. Data from 1981 are presented in a; data from current reporting period are in b. Zone interpretations are 14 or less = resistant (R), and 15 or greater = sensitive (S). Intermediate (I) interpretation is for zones greater than 14 and less than 15.



.2 H_2O

CHLORHEXIDINE DIPHOSPHANILATE DIHYDRATE

WP-973 (WESTWOOD)

Figure 2. Chemical structure of chlorhexidine diphosphanilate dihydrate.

into four groups. One group was given one human dose of the vaccine (i.p.) 7 days prior to burning. A second group was given one human dose (i.p.) 7 days prior and again 1 day prior to burning. Control groups were given saline i.p. at 7 days prior to burning. The two vaccinated groups and one control group were burned and infected with P. aeruginosa strain 59-1244. The second control group was burned and not infected. Animals were observed for 21 days. Results are presented in Table 3. As can be seen, a single injection with one human dose was not significantly effective, but two injections improved survival ($P < 0.01$).

IN VITRO SENSITIVITY TO SULFAMYLON OF PSEUDOMONAS AERUGINOSA RECOVERED FROM BURN PATIENTS

In FY 82, 735 strains of P. aeruginosa were tested for in vitro sensitivity to Sulfamylon acetate. Tests were conducted using the previously reported (Lindberg, USAISR Annual Report, 1965) agar dilution assay. Organisms were tested for sensitivity to 5% = 5 gm/dl through -.019%. Data are reported as the lowest concentration of Sulfamylon to inhibit growth of a 1,000 organism inoculum at 24 hours of incubation. The distribution of sensitivities is presented in Table 4. The average minimal inhibitory concentration (MIC) was 0.366% and the median MIC was 0.295%. A 10-year summary of P. aeruginosa sensitivity to Sulfamylon is presented in Table 5.

PRESENTATIONS

McManus AT: Efficacy of cefsulodin (CGP7.1743) in experimental Pseudomonas burn wound sepsis in the rat. Sixth International Congress on Burns, International Society for Burn Injuries, San Francisco, California, 3 September 1982.

Table 3. Evaluation of Wellcome Polyvalent Pseudomonas Vaccine in Burned Rats

Group	Survival[1]
Group 1 1 human dose day -7 (i.p.) infected	7/19*
Group 2 2 human doses day -7, -1 (i.p.) infected	14/18**
Group 3 saline (i.p.) infected	2/19
Group 4 saline (i.p.) not infected	18/20

[1] All groups burned. Groups 1, 2 and 3 infected with strain 59-1244 following burns. Survival was measured at 21 days postburn.

* Group 1 vs. Group 3, N.S., $P = 0.06$.

** Group 2 vs. Group 3, improved survival, $P < 0.001$.

Table 4. Sensitivity of Pseudomonas aeruginosa to Sulfamylon
1 October 1981 - 30 September 1982

No. of Strains	Concentration Required for Inhibition (gm/dl)	% of Total Tested
2	1.250	0.27
348	0.625	47.35
116	0.312	15.78
40	0.156	5.44
50	0.078	6.80
148	0.039	20.14
31	0.019	4.22
0	< 0.019	0

Table 5. Median Value of Pseudomonas aeruginosa Sensitivity to Sulfamylon, 1972-1982

Year	No. of Strains Tested	Median Inhibitory Level (gm/dl)
1972	463	0.316
1973	285	0.111
1974	437	0.086
1975	656	0.125
1976-77	698	0.117
1977-78	141	0.089
1978-79	715	0.324
1979-80	461	0.198
1980-81	468	0.253
1981-82	733	0.295

TERMINATION REPORT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

REPORT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS -- A CLINICAL STUDY TO ASSESS THE SAFETY AND EFFICACY OF ABBOTT-46811 IN THE TREATMENT OF INFECTIONS CAUSED BY PSEUDOMONAS AERUGINOSA IN BURN PATIENTS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1981 - 30 September 1982

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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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REPORT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS - A CLINICAL STUDY TO ASSESS THE SAFETY AND EFFICACY OF ABBOTT-46811 IN THE TREATMENT OF INFECTIONS CAUSED BY PSEUDOMONAS AERUGINOSA IN BURN PATIENTS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

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William F. McManus, M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

The third generation cephalosporin cefsulodin sodium (showing excellent activity against Pseudomonas organisms resistant to either aminoglycosides or the semi-synthetic penicillins) was evaluated in the treatment of sepsis caused by Pseudomonas organisms in burn patients. Ten patients met the entry criteria, with six patients receiving cefsulodin while two patients received ticarcillin and two patients amikacin therapy. In the cefsulodin treatment group, five patients had Pseudomonas pneumonia and one had a Pseudomonas burn wound infection. Four of the cefsulodin treated patients expired, with the duration of treatment ranging from 1 to 14 days. A positive treatment effect was observed in four cefsulodin treated patients on the basis of survival of two and clearing of the causative organisms in two others. No adverse reactions were observed in the four patients receiving cefsulodin for periods of 7 to 14 days. Three of the four patients receiving treatment with reference drugs expired.

Cefsulodin appears to show a high degree of effectiveness against Pseudomonas organisms with no renal toxicity or microbial resistance identified in the study patients. In those patients who survived following a diagnosis of Pseudomonas wound infection, cefsulodin therapy was adjunctive, since early excision of infected tissue was also carried out.

Infection
Antibiotic effects
Pseudomonas
Humans

ALTERATIONS OF HOST RESISTANCE IN BURNED SOLDIERS - A CLINICAL STUDY TO ASSESS THE SAFETY AND EFFICACY OF ABBOTT-46811 IN THE TREATMENT OF INFECTIONS CAUSED BY PSEUDOMONAS AERUGINOSA IN BURN PATIENTS

All of the available topical burn wound antimicrobial agents are imperfect and are incapable of preventing invasive burn wound infection in certain patients with extensive burns (1). The flora of burn wounds changes with time following injury and by the second postburn week is predominantly gram-negative, with Pseudomonas organisms prominent in the population (2). Even in the absence of frank wound infection, systemic infections are common as a result of periodic bloodstream seeding due to wound manipulation and the immunosuppressive effects of the burn injury (3,4). The use of aminoglycosides has been associated with the development of a resistance to such agents in the Pseudomonas organisms causing burn wound sepsis and those recovered from the blood of burn patients with other infections (5). The frequency of sepsis caused by Pseudomonas organisms resistant to available antibiotics has prompted a clinical evaluation of the effectiveness and safety of a new third generation cephalosporin in the treatment of such infections.

MATERIALS AND METHODS

Cefsulodin sodium (Abbott-46811) is the monosodium salt of a carboxylated sulfophenylacetamido compound which has marked activity against Pseudomonas aeruginosa. It also shows activity against Staphylococci, beta hemolytic Streptococci, pneumococci, and Neisseria but shows little if any activity against Enterobacteriaceae. Of particular interest is the fact that the compound has shown excellent in vitro activity against Pseudomonas organisms resistant to either aminoglycosides or the semisynthetic penicillins (6).

---

1. Pruitt BA, Jr and Curreri PW: The burn wound and its care. Arch Surg 103:461-468, 1971.

2. Pruitt BA, Jr and Lindberg RB: Pseudomonas aeruginosa infections in burn patients. In Pseudomonas aeruginosa, Clinical Manifestations of Infection and Current Therapy, R.G. Doggett (ed.), Academic Press, New York, 1979, pp. 339-366.

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4. Pruitt BA, Jr: The burn patient: II. Later care and complications of thermal injury. In Current Problems in Surgery, M. Ravitch (ed.), Vol. XVI, No. 5, Year Book Medical Publishers, Chicago, 1979, pp. 45-52.

5. Pruitt BA, Jr: Infections of burns and other wounds caused by Pseudomonas aeruginosa. In Pseudomonas aeruginosa, L.D. Sabath (ed.), Hans Huber Publishers, Berne, 1980, pp. 55-70.

6. Cefsulodin Sodium (Abbott-46811) Information for Clinical Investigators, Abbott Laboratories, Pharmaceutical Products Division Research and Development, North Chicago, Illinois, 1980, pp. II-1 to III-13.

Male and female burn patients of more than 16 years with Pseudomonas cultured from at least one site, the burn wound, sputum, urine, or blood, in whom the organism was sensitive to both cefsulodin sodium and at least one other available antimicrobial agent were included in the study. The patients were randomly assigned to receive either cefsulodin 1 gm IV q 6 hours or a standard recommended dose of the reference drug for a period of 10 to 14 days. The course of each patient was subsequently assessed in terms of vital signs, clinical status, laboratory profile, and appropriate cultures and sensitivities.

RESULTS AND DISCUSSION

Ten patients meeting the entry criteria were studied during the current fiscal year (Table). Six patients of 16 to 65 years with burns ranging from 26.5% to 80% of the total body surface received cefsulodin while two patients received ticarcillin and two patients amikacin therapy. In the cefsulodin treatment group, five patients has Pseudomonas pneumonia documented by culture and one had a Pseudomonas burn wound infection. Four of the patients treated with cefsulodin expired from one to 26 days following the initiation of treatment. The duration of treatment ranged from one to 14 days. The effect of treatment was deemed to be positive in four patients on the basis of survival in two and clearing of the causative organism as determined by culture in two other patients who expired 12 and 26 days after initiation of cefsulodin treatment. The treatment effect was considered indeterminate in two patients who expired 24 and 48 hours after initiation of treatment. No adverse reactions were observed in four patients who received cefsulodin treatment for a period of 7 to 14 days. Assessment of adverse reactions was considered indeterminate in the two patients who received treatment for only 24 and 48 hours, respectively.

One patient who received ticarcillin survived and was discharged from the hospital on the 84th postburn day, having shown a positive treatment effect and no adverse reactions. The other ticarcillin patient died on the 213th day from another infection and showed the development of ticarcillin resistance in those Pseudomonas organisms causing persistent and recurrent sepsis. Both of the amikacin-treated patients expired, one of whom showed renal toxicity manifested by a rising BUN following four days of amikacin treatment. The other amikacin-treated patient expired 24 hours following the initiation of amikacin therapy for treatment of invasive burn wound sepsis.

In summary, cefsulodin appears to be safe and to show a high degree of effectiveness against Pseudomonas organisms causing either pneumonia or wound infections in burn patients as indicated by clearing of cultures in the four patients receiving treatment for more than 48 hours. Contrary to the experience in the small number of patients treated with either ticarcillin or amikacin, no incidence of renal toxicity or development of microbial resistance was identified in the patients treated with cefsulodin. The narrow spectrum of activity of this agent and the sensitivity to cefsulodin of the Pseudomonas flora causing infections in

these burn patients recommend its use in the treatment of documented Pseudomonas infections in the burn population. It should be noted that in the patients in this study who survived following the diagnosis of Pseudomonas wound infection, the diagnosis was made in a timely fashion and early excision of the infected tissue was carried out with antimicrobial treatment being adjunctive (7).

The manufacturer of cefsulodin sodium has terminated this study because of the small number of patients entered in this multi-center open parallel study and the irregular quality of case reports received from other institutions.

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(7) McManus WF, Goodwin CW, Mason AD, Jr, Pruitt BA, Jr: Burn wound infection. J Trauma 21:753-756, 1981.

PUBLICATIONS/PRESENTATIONS

None.

SUMMARY TABLE: CLINICAL STUDY OF CEFSULODIN SODIUM

Antibiotic	Age	Sex	% TBS	% 3rd°	Type Infection	PBD Inf Treated	Treatment Days	Treatment Effect	Adverse Reaction	Outcome/PBD
Cefsulodin	62	M	58.5	2.5	Pneumonia	11	1	Ind	Ind	Death/12
	28	M	53.0	29.0	Pneumonia	12	7	Pos	None	Death/34
	16	M	80.0	75.0	Wound	11	10	Pos	None	Disch/159
	65	M	38.0	0.0	Pneumonia	26	10	Pos	None	Death/38
	59	M	47.5	16.5	Pneumonia	9	14	Pos	None	Disch/70
	57	M	26.5	12.0	Pneumonia	16	2	Ind	Ind	Death/18
Ticarcillin	60	M	29.0	8.3	Pneumonia	29	6	Pos	None	Disch/84
	81	F	22.5	16.0	Pneumonia	54	8	Neg	Resistance	Death/213
Amikacin	19	M	77.0	39.0	Wound	18	4	Neg	Renal tox	Death/23
	55	M	45.0	3.0	Wound	6	1	Ind	Ind	Death/7

Ind - Indeterminate

TERMINATION REPORT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

REPORT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS - INCREASED SUSCEPTIBILITY TO INFECTION RELATED TO EXTENT OF BURN. INVASION OF PARTIAL THICKNESS BURN WOUNDS BY PSEUDOMONAS AERUGINOSA, STRAIN 59-1244

US ARMY INSTITUTE OF SURGICAL RESEARCH
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Reports Control Symbol MEDDH-288(R1)

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ABSTRACT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

REPORT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS - INCREASED SUSCEPTIBILITY TO INFECTION RELATED TO EXTENT OF BURN. INVASION OF PARTIAL THICKNESS BURN WOUNDS BY PSEUDOMONAS AERUGINOSA, STRAIN 59-1244

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1981 - 27 July 1982

Investigators: Roger W. Yurt, M.D., Major, MC
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Previous reports from this laboratory have documented a model of burn wound sepsis in the rat. This report confirms those observations and indicates that partial thickness wounds of the same size are not susceptible to burn wound invasion, however, if the extent of burn injury is increased the partial thickness wound becomes invaded with bacteria with resulting increased mortality. The data presented confirm the hypothesis that extent of injury is associated with susceptibility to infection.

Burn
Infection

ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS - INCREASED SUSCEPTIBILITY TO INFECTION RELATED TO EXTENT OF BURN. INVASION OF PARTIAL THICKNESS BURN WOUNDS BY PSEUDOMONAS AERUGINOSA, STRAIN 59-1244

INTRODUCTION

The development of a reproducible model of burn injury (1) coupled with the subsequent establishment of a model of burn wound sepsis in the rat has provided a means to evaluate the pathogenesis (2) and therapy (3) of burn injury. Nevertheless, previous animal models of burn injury have not accounted for the variable outcome among patients with similar injuries and the observed increased suspectibility to infection in those with larger injuries. In an effort to evaluate the mechanisms of varying response to injury and infection, a partial thickness burn wound model has been developed in the rat. Inoculaton of a thirty percent body surface area full thickness injury with Pseudomonas aeruginosa leads to infection of the burn wound and 100% mortality. In contrast, partial thickness wounds of the same size are resistant to the same inoculum. However, if rats sustain an additional 30% full thickness injury, the 30% partial thickness wound becomes susceptible to microbial invasion and 50% of the inoculated rats succumb to sepsis. This report supports this injury as a model of depressed resistance to infection and confirms the hypothesis that increase in extent of injury leads to increase in susceptibility to infection.

MATERIAL AND METHODS

Sprague-Dawley rats weighing 350 grams were used in all experiments. Each rat received 0.5 ml of pentobarbital (25 mg/kg) intraperitoneally prior to burn injury. Rats were placed

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1. Walker HL and Mason AD Jr.: A Standard Animal Burn. J. Trauma 1968; 8:1049-1051
2. Teplitz C, Davis D, Mason AD Jr., and Moncrief JA: Pseudomonas Burn Wound Sepsis. I. Pathogenesis of Experimental Pseudomonas Burn Wound Sepsis. J.S.R. 1964; 4:200-216
3. Lindberg RB, Moncrief JA, and Mason AD Jr.: Control of Experimental and Clinical Burn Wound Sepsis by Topical Application of Sulfamylon Compounds. Ann. N.Y. Acad. Sci. 1968; 150:950

in molds so as to expose 30% of the total body surface to 95°C water (1). Contact of the rat's dorsal surface with the water for 2 or 10 seconds led to partial thickness or full thickness injury, respectively. Two second exposure of the ventral surface produced full thickness injury. In each case after dorsal injury, the rat received 15 cc or 30 cc of saline when the total extent of injury was 30 to 60%, respectively. The rat was repositioned to allow ventral exposure to 95°C water or nothing (sham). Rats that were inoculated had 1 ml of medium containing 10^8 colony forming units (CFU) of Pseudomonas aeruginosa strain 59-1244 spread over their dorsal wounds within 30 minutes after burn injury as previously described (3). Quantitative wound biopsy, organ, and blood culture were performed as described (4) at autopsy or at specified times. Prospective evaluation of rats was performed by daily observation for clinical signs and wound changes.

RESULTS

In an initial experiment, 16 rats sustained 30% full (ventral) and 30% partial (dorsal) burns. The partial thickness wounds of eight rats were inoculated with Pseudomonas 59-1244 immediately after injury and eight rats were not inoculated. By the twelfth post injury day, there was a 50% mortality in the inoculated group and no mortality in those without inoculation. There were no additional deaths after 28 days post burn. In contrast eight rats that sustained only 30% partial thickness (dorsal) burn with Pseudomonas inoculation had only a 12.5% mortality (1 death on post burn day 7) over the duration of the experiment. Six additional rats in this experiment sustained 30% full thickness burns (dorsal) and were inoculated. The mortality of 100% in this group of rats by 12 days is consistent with previous reports (2) of mortality in full thickness burns inoculated with Pseudomonas aeruginosa strain 59-1244.

In order to confirm these results, this experiment was repeated in the same manner except that the 30% full and 30% partial thickness uninoculated group was not included. During the first 11 days post burn, there was again a 50% mortality in the rats sustaining 30% full (ventral) and 30% partial thickness (dorsal) burns with inoculation of the partial thickness wound. One additional rat died in this group at 20 days post injury. As in the previous experiment one out of eight rats with 30% partial thickness inoculated wound died (post burn day 14). By post burn day 10, the mortality was

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4. McManus AT, McLeod CG Jr., and Mason AD Jr.: Experimental Proteus mirabilis Burn Surface Infection. Arch. Surg. 1982; 117:187-191

100% in 6 rats that sustained 30% full thickness burns and were inoculated. This experiment, therefore, almost exactly duplicated the results of the initial experiment. A summary of the mortality in both experiments combined is shown in Table 1.

Confirmation of the depth of injury was obtained by clinical observation and evaluation of wound histology. The wounds of rats (n=8) sustaining 30% full (ventral) and 30% partial thickness (dorsal) burns were observed for 28 days. At two weeks the dorsal surface had 27.5 $\pm$ 0.77% partial thickness injury while the ventral surface was all full thickness. By four weeks all wounds were healed; the partial thickness by epithelization and full thickness by this process and contraction. Survivors of 30% partial thickness (dorsal) injury plus inoculation with Pseudomonas (n=7) had 26.6 $\pm$ 1.6% partial thickness wound at two weeks post injury; all of which were healed at four weeks. The uninoculated dorsal burns were partial thickness as confirmed by microscopic evaluation of biopsies taken from rats at four hours after 30% (n=5) and 60% (n=5) and 8 hours after 30% (n=10) and 60% (n=10) burn.

The wounds of nonsurviving rats with partial thickness inoculated injury appeared to convert to full thickness injury prior to death. In order to verify this observation and determine if the post burn course was consistent with progressive sepsis, a prospective study of clinical signs, weights, and wound changes was performed. Rats were observed throughout the 28 day post injury course. Clinical evaluation included observations of respiratory changes (primarily palpable respiratory change and/or bloody nasal discharge), fluffy hair, hemorrhagic conjunctivitis, and lethargy. Three out of four nonsurvivors in the inoculated 60% burn group developed respiratory change and hemorrhagic conjunctivitis. These findings paralleled those of the six 30% full thickness burned nonsurvivors where five had respiratory change and four had hemorrhagic conjunctivitis. Survivors in the 30% full thickness and 30% partial thickness burned and inoculated group had no adverse clinical findings except that 2 out of 4 had transient respiratory changes. In addition the nonsurviving rats with 30% full thickness and 30% partial thickness inoculated wounds followed a septic course similar to the 30% full thickness inoculated nonsurvivors with regard to weight changes (Table 2). Weights reported as a fraction of initial weight decreased over the two to three days prior to death in both groups. Although surviving inoculated 60% burned rats did not gain weight, their weight did remain stable.

These findings suggested that sepsis was the cause of death in the rats with 30% full thickness plus 30% partial

TABLE 1. Relationship Between Extent of Injury and Mortality of Rats with or without Inoculation of Wounds with Pseudomonas aeruginosa Strain 59-1244

Extent of Burn	Depth of Burn Inoculated	N	Days Post Burn										Mortality
			4	6	7	8	9	10	11	12	14	28	
30% Partial	Partial	16	0[1]	0	1	0	0	0	0	0	1	0	12.5%
30% Full	Full	12	1	0	0	3	5	2	0	1	0	0	100.0%
30% Partial, 30% Full	Partial	16	0	1	1	1	1	1	2	1	0	1[2]	56.3%
30% Partial, 30% Full	------	8	0	0	0	0	0	0	0	0	0	0	0.0%

[1] Number of rats that died

[2] Died on day 20

Table 2. Comparison of Weight Change in Surviving and Nonsurviving Rats after 30 or 60% Burn and Inoculation with Pseudomonas aeruginosa Strain 59-1244

Extent of Burn	N	Days Prior to Death 5	4	3	2	1
30%[1]	6	0.932 ± 0.07	0.897 ± 0.086	0.903 ± 0.102	0.852 ± 0.09	0.794 ± 0.066
60%[2]	4	0.982 ± 0.05	0.946 ± 0.049	0.898 ± 0.031	0.837 ± 0.01	0.757 ± 0.041
		Time Matched Surviving Rats				
60%[2]	4	0.996 ± 0.102	0.998 ± 0.007	0.990 ± 0.077	0.986 ± 0.091	0.989 ± 0.066

[1] Full Thickness

[2] 30% Partial, 30% Full Thickness
Fraction of Preburn Weight ± SEM

thickness inoculated burns. However, the possibility remained that the seeded partial thickness dorsal wounds led to contamination and invasion of bacteria into the full thickness ventral injury. That the dorsal partial thickness wound was the primary site of infection was suggested by changes in the wounds. Over the five days prior to death in four rats, the ventral full thickness wounds contained stable amounts of hemorrhage with 2 wounds showing transient greenish discoloration and one with localized black discoloration. The findings in the dorsal wounds were consistent with progressive conversion showing little hemorrhage early and more prominent hemorrhage associated with edema at death. All dorsal wounds had a green appearance and in 2 progressed to black discoloration 2 days prior to death. That in a majority of cases the primary infection was in the partial thickness wound was confirmed by the results of quantitative culture of biopsies of both the ventral and dorsal wounds of rats dying within the first 12 days after injury (Table 3). Three out of four rats had greater than 10^6 Pseudomonas aeruginosa per gram of dorsal skin while only one of four ventral biopsies had greater than 4×10^4 organisms per gram of tissue. Several biopsies contained Proteus sp. in addition to Pseudomonas aeruginosa and one ventral biopsy contained only Proteus sp. That septicemia was present was suggested by the finding of Pseudomonas in all hearts and spleens with the exception of one which grew only Proteus sp. These findings were consistent with those in the rats that sustained 30% full thickness injury plus inoculation with Pseudomonas aeruginosa. At autopsy all these rats had more than 10^5 Pseudomonas per gram of wound and all of the 5 spleens and hearts tested grew Pseudomonas aeruginosa.

To further evaluate the development of sepsis and confirm bacteremia, 6 rats sustained 30% full thickness ventral and 30% partial thickness dorsal burns. The dorsal wounds were inoculated with Pseudomonas aeruginosa immediately after burn and the rats were sacrificed at 6, 8, and 10 days post injury. After induction of anesthesia on the prescribed day, blood culture and wound biopsies were obtained in each case (Table 4). One rat died of Pseudomonas sepsis (positive culture of heart and spleen) on day 7 post burn and was excluded. The bacterial count was higher in the dorsal than in the ventral biopsy except in one animal, where the ventral had three times more than the dorsal. All blood cultures were positive for Pseudomonas and two rats in addition had Proteus sp. in their blood.

Table 3. Comparison of Quantitative Wound Biopsy Culture of 30% Dorsal Partial and 30% Ventral Full Thickness Wounds and Cultures of Viscera at Autopsy

Day Post Burn	Ventral		Dorsal		Heart and Spleen Organism
	CFU[2]/Gram	Organism	CFU/Gram	Organism	
7	4×10^4	Ps, P	3.3×10^6	Ps	Ps[1]
9	2×10^4	Ps, P	6.0×10^6	Ps, P	Ps, P
11	1×10^4	P	1.2×10^7	Ps, P	P
11	2×10^6	Ps	2.0×10^1	Ps	Ps, P

[1] Ps = Pseudomonas aeruginosa, P = Proteus species

[2] Colony forming unit

Table 4. Results of Prospective Evaluation of 30% Dorsal Partial and 30% Ventral Full Thickness Wounds by Quantitative Culture of Wound Biopsies

Day Post Burn	Ventral		Dorsal		
	CFU/Gram	Organism	CFU/Gram	Organism	Blood Culture
6	1.7×10^4	Ps[1]	1.5×10^7	Ps	Ps, P
6	1.0×10^4	Ps	4.0×10^4	Ps	Ps, P
8	1.0×10^3	Ps	1.5×10^4	Ps	Ps
10	3.0×10^6	Ps	1.0×10^6	Ps	Ps
10	3.0×10^2	Ps	1.0×10^4	Ps	Ps

[1] Ps = Pseudomonas aeruginosa, P = Proteus species

DISCUSSION

Thirty percent partial thickness wounds in rats are resistant to invasion by Pseudomonas aeruginosa strain 59-1244 and the mortality from such wounds, even with seeding, is low. However, in the presence of additional uninoculated wound, the partial thickness injury becomes invaded, leading to increased mortality (Table 1). Although varying amounts of full thickness injury or subsequent conversion of partial thickness burn might account for heightened microbial invasion, histological data confirm that the wounds were partial thickness and clinical evaluation confirmed that uninoculated wounds healed whether the rat sustained a 30 or 60% surface area burn. In addition the inoculated wounds of survivors of 30% partial thickness burns showed no conversion to deeper injury.

That the mortality in the inoculated rats was due to sepsis is supported by clinical findings including the development of respiratory changes, hemorrhagic conjunctivitis, weight loss and progressive wound changes. These findings were similar in rats dying after 30% full and 30% inoculated partial thickness inoculated burns and rats dying after 30% full thickness inoculated burns except that the partial thickness wound appeared to convert to deeper wound as infection progressed. In addition when rats were sacrificed at intervals after 30% full and 30% partial thickness, inoculated wounds, all were bacteremic, including those sampled at 6 days post injury (Table 4). Furthermore, all rats tested that died had positive cultures of heart and spleen.

The primary source of sepsis appeared to be the partial thickness wounds in the rats sustaining 60% burns since the autopsy data in 3 out 4 cases revealed quantitative bacterial counts of at least 10^6 per gram in the partial thickness wounds. In contrast only one rat had quantitative counts this high in full thickness wound; all other full thickness wounds had counts a hundred to a thousand fold lower. The progressive changes in the partial thickness wounds supported the bacteriologic findings that these wounds rather than the full thickness injuries were the source of sepsis. Taken together the data support the clinical impression that extent of injury is a determinant of mortality due to infection and that resistance to infection is more compromised after extensive injury. More specifically, partial thickness burns are less resistant to microbial invasion when additional full thickness injury is present.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA 0G 6972	2. DATE OF SUMMARY: 82 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR)636
3. DATE PREV SUM'RY: 81 10 01	4. KIND OF SUMMARY: D. CHANGE	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING:	8a. DISP'N INSTR'N: NL	8b. SPECIFIC DATA-CONTRACTOR ACCESS: ☒ YES ☐ NO	9. LEVEL OF SUM: A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BB	301
b. CONTRIBUTING				
c. XXXXXXXXX	STOG 80 -	7.2:5		

11. TITLE (Precede with Security Classification Code)

(U) Studies of Infection and Microbiologic Surveillance of Troops With Thermal Injury

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 10	Cont	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	2.5	200
	CURRENT 83	2.5	246

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

ADDRESS: Fort Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL

NAME: Basil A. Pruitt, Jr., MD, COL, MC

TELEPHONE:

20. PERFORMING ORGANIZATION

NAME: US Army Institute of Surgical Research
Microbiology Branch

ADDRESS: Fort Sam Houston, Texas 78234

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: Albert T. McManus, GM-15

TELEPHONE: 512-221-3411

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME: POC: DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Pseudomonas; (U) Klebsiella; (U) Staphylococcus; (U) Wound Infection; (U) Antibiotic Resistance; (U) Sepsis, (U) Topical Chemotherapy; (U) Humans

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Burns constitute a large component of military injuries sustained in combat. Military relevance of this research lies in the fact that infection and ensuing sepsis are major problems among burned soldiers. Control of surface infection is a major objective, and species of organisms causing sepsis, epidemiology, response of significant species to topical chemotherapy modalities, and relation of antibiotics to sepsis control are major study areas.

24. (U) Culture of human wounds, tissues and body fluids are carried out with precise strain speciation and differentiation being employed. Virulence is assessed in burn wound models which are also used to assess effectiveness of experimental drugs, both topical and systemic.

25. (U) 8110 - 8209. Pseudomonas aeruginosa was the most common burn isolate (1116 strains). This was followed in descending frequency by: Providencia stuartii (899), Staphylococcus aureus (773), Klebsiella pneumoniae (486), Streptococcus viridans (452), Candida sp.(395), Escherichia coli (299), Group D Enterococcus (274), non-hemolytic, not Group D Streptococcus (250). These eight organisms represented more than 75% of all isolates. Blood cultures were positive for 256 of 2,154 cultures. Positive cultures were found in 73 of 179 cultured patients. The principal blood isolates were: Providencia stuartii (26 patients), Staphylococcus aureus (22 patients), Pseudomonas aeruginosa (20 patients), yeast sp. (19 patients) and Group D Enterococcus (15 patients). An additional 17 species were isolated from blood cultures.

*Available to contractors upon originator's approval

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ANNUAL PROGRESS REPORT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1981 - 30 September 1982

Investigators

Albert T. McManus, Ph.D., Major, MSC
Jack R. Henderson, Ph.D.
Tommy C. Alderson, SSG
Lidia A. Brownell, SP5
Timothy E. Lawson, SP5
Aldo H. Reyes, SP5

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY

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Pseudomonas aeruginosa was the most common burn isolate (1116 strains). This was followed in descending frequency by: Providencia stuartii (899), Staphylococcus aureus (773), Klebsiella pneumoniae (486), Streptococcus viridans (452), Candida sp. (395), Escherichia coli (299), Group D Enterococcus (274), non-hemolytic, not Group D Streptococcus (250). These eight organisms represented more than 75% of all isolates. Blood cultures were positive for 256 of 2,154 cultures. Positive cultures were found in 73 of 179 cultured patients. The principal blood isolates were: Providencia stuartii (26 patients), Staphylococcus aureus (22 patients), Pseudomonas aeruginosa (20 patients), yeast sp. (19 patients) and Group D Enterococcus (15 patients). An additional 17 species were isolated from blood cultures.

Pseudomonas
Klebsiella
Staphylococcus
Wound infection
Antibiotic resistance
Sepsis
Topical chemotherapy
Humans

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY

An attempt has been made to combine antimicrobial susceptibility testing and surveillance culture data into an ongoing automated data base. The prospective clinical utility of an automated microbiology data base is dependent on several assumptions. It can be assumed that infections in burned patients are not spontaneous; rather, they are the eventual result of inappropriate growth of endogenous flora or the result of colonization with acquired organisms that progressed to infection in the presence of decreased host resistance. Under either scenario, a time window should exist between patient injury and infection. A further assumption is that a protocol can be established to monitor sites of potential infection and allow isolation and characterization of potential opportunistic pathogens prior to infection. Collection of such data and addition to a cumulative data base on an individual basis would, in theory, document the natural history of each patient's infection. It may also be possible to identify risk factors and bacteriological events that will act as predictors or maximum likelihood indicators for selection of appropriate therapeutic antimicrobial agents.

Captain Steven G. Fehrman (Biomedical Information Officer) was instrumental in the development of this microbiology data system. He provided the many necessary programming and communication skills to convert routine laboratory and patient census data into an ongoing patient reporting and summarisation system.

ANTIBIOTIC SENSITIVITY DETERMINATION

A technical change has been made in routine antimicrobial sensitivity testing. Tube dilution minimal inhibitory concentration testing of antibiotics is no longer the principal method. Testing is now done by disc diffusion zone of inhibition technique. Agar overlay disc tests are performed at 35° C using the methods and interpretation of the National Committee for Clinical Laboratory Standardization. Bacterial isolates were tested on the following protocol: blood culture isolates, predominant organisms in biopsy specimens, predominant organisms in urine growing $\geq 10^5$ CFU/ml, predominant gram-negative isolates in upper respiratory specimens, Staphylococcus aureus isolates, Pseudomonas aeruginosa isolates and any other isolates requested. Antibiotic batteries for gram-negative and gram-positive organisms are presented in Table 1.

DAILY REPORTING

Culture specimens submitted for examination are assigned accession numbers and processed by standard laboratory procedures. Following 24-hour or longer incubation, specimen growth results are logged into the computer. Cultures are identified by accession number, patient name, source name, date taken, date received in the laboratory and patient ward location. On a daily basis, results of pending cultures are updated and a written report printed. An example of a daily clinical microbiology report is presented

Table 1. Antibiotics Used for Sensitivity Testing

GRAM-POSITIVE ORGANISMS			GRAM-NEGATIVE ORGANISMS		
ANTIBIOTIC	SYMBOL	DISC CONCENTRATION	ANTIBIOTIC	SYMBOL	DISC CONCENTRATION
AMIKACIN	AM	30 µg	AMIKACIN	AM	30 µg
GENTAMICIN	GM	10 µg	GENTAMICIN	GM	10 µg
TOBRAMYCIN	NN	10 µg	TOBRAMYCIN	NN	10 µg
TICARCILLIN	TIC	75 µg	TICARCILLIN	TIC	75 µg
MEZLOCILLIN	MZ	75 µg	MEZLOCILLIN	MZ	75 µg
PIPERACILLIN	PIP	100 µg	PIPERACILLIN	PIP	100 µg
METHICILLIN	DP	5 µg	MOXALACTAM	MOX	30 µg
CEPHALOTHIN	CF	30 µg	CEFOTAXIME	CTX	30 µg
MOXALACTAM	MOX	30 µg	CEFSULODIN*	CEF	30 µg
CEFOTAXIME	CTX	30 µg	CEFOPERAZONE*	CFP	75 µg
VANCOMYCIN	VA	30 µg	COLISTIN	CO	10 µg
SULFADIAZINE	SD	250 µg	SULFADIAZINE	SD	250 µg

* Investigational new drugs

in Table 2. Sensitivity testing on an isolate is indicated by an asterisk preceding the organism name. Sensitivities are reported as sensitive, intermediate sensitive or resistant to a particular antibiotic as indicated by manufacturer's zone interpretation guidelines. Sensitivity data are entered into the computer as inhibition zone diameters in millimeters. Antibiotic sensitivity reports are printed with the daily clinical microbiology reports as shown in Table 3.

SUMMARY REPORTING

Data may be summarized between designated dates. This may be for a patient, a ward, a type of organism, a culture source, or for several simultaneous variables. An actual example of a patient record summary during intensive care is presented in Table 4. Data are listed in chronological order from patient admission to transfer from intensive care. The heading of WARD indicates location ward: 1 shows the patient's location as the intensive care unit, Ward 14A. DTAKEN indicates the date of specimen collection. A specimen without an organism name listed indicates no growth from that specimen. Q1 and Q2 are codes for quantitative results. A count of 2×10^6 organisms would be printed as Q1=2 and Q2=6. AS codes for antibiotic sensitivity testing; an asterisk indicates testing has been done on a particular organism. Patient summaries may be printed after sorting the data by any variable heading for which there are data entered. For example, Table 5 displays the patient's record sorted by organism type. No name under organism name indicates a negative specimen. This display may be useful in accessing the temporal relationship of organisms and sites of isolation. This patient had a Pseudomonas aeruginosa bacteremia on 30 August 1982. Pseudomonas aeruginosa had been previously isolated in the urine on 18 and 20 August and on wound contact plate on 20 August. The similarities of these Pseudomonas aeruginosa isolates will be described below.

Antibiotic sensitivity testing data may also be summarized. The summary of this patient's Pseudomonas aeruginosa isolates is presented in Table 6. In this display, intermediate sensitive and sensitive results are reported as S. The patient's blood isolate was resistant to tobramycin and sulfadiazine. The two previous urine isolates match this pattern. The wound isolate had additional resistance to moxalactam and cefotaxime and thus was not identical to the blood isolate. The zone diameters used for sensitivity interpretation of these isolates are presented in Table 7. These data again show the similarity of the blood isolate and the urine isolates. Serologic typing also showed these organisms to be of the same O-type.

These promising techniques are being further developed. A clinical infection monitoring program is being developed in cooperation with the infection control nurse and the Chief of the Clinical Division. This program will dovetail with the microbiology data base. This combined file will be used to test specific hypotheses regarding sources of infection. Results will be presented in future reports.

TOTAL BURN PATIENT MICROBIOLOGY SUMMARY

During this reporting period, 7177 specimens were submitted from a total of 216 burned patients. The distribution of specimen types is presented in

Table 2

US ARMY INSTITUTE OF SURGICAL RESEARCH

DAILY CLINICAL MICROBIOLOGY REPORT

Date: 26 DEC 81
Day : SATURDAY

ISR#, PATIENT TAKEN: Date,Time RECD : Date,Time Assn Number	SOURCE Source Description QUANTY	DIAGNOSIS sensitivities done - * # *
000-81 SMITH, JOHN Taken:17-DEC-81 , 0600 Recd :17-DEC-81 , Assn :81-12-17-05	BLOOD	NO GROWTH IN 10 DAYS
000-81 SMITH, JOHN Taken:23-DEC-81 , 0300 Recd :23-DEC-81, Assn :81-12-23-02	SPUTUM 2.3X10^5	G N ENTERO,# S AUREUS ,
000-81 DOE, JOHN Taken:17-DEC-81 , 0600 Recd :17-DEC-81 , Assn :81-12-17-01	BLOOD POSITIVE ((AEROBIC #))	# P AERUG ,
000-81 DOE, JANE Taken:24-DEC-82 , 0500 Recd :24-DEC-82, Assn :82-12-24-07	URINE 1.0X10^3	# E COLI ,
000-81 DOE, JANE Taken:24-DEC-82 , 0500 Recd :24-DEC-82, Assn :82-12-24-13	SPUTUM 2.3X10^5	# K PNEUMO , S VIRID ,

Table 3

DAILY CLINICAL MICROBIOLOGY REPORT

* Antibiotic Sensitivities *

Date: 26 DEC 81
Day : SATURDAY

ISR# , PATIENT TAKEN: Date,Time Assn Number	SOURCE QUANTY	ORGANISM	ANTIBIOTIC SUSCEPTIBILITIES
000-81 SMITH, JOHN Taken:23-DEC-81 , 0300 Assn:81-12-23-02	SPUTUM 2.3X10^5	GRAM POS S AUREUS	AMIKACIN - SEN GENTAMICIN - SEN TOBRAMYCIN - SEN TICARCILLIN - SEN MEZLOCILLIN - SEN PIPERACILLIN - SEN METHICILLIN - SEN CEPHALOTHIN - SEN MOXALACTAM - RES CEFOTAXIME - SEN VANCOMYCIN - SEN SULFADIAZINE - SEN
000-81 DOE, JOHN Taken:17-DEC-81 , 0600 Assn:81-12-17-01	BLOOD POSITIVE	PSEUDO P AERUG	AMIKACIN - SEN GENTAMICIN - IN TOBRAMYCIN - SEN TICARCILLIN - SEN MEZLOCILLIN - RES PIPERACILLIN - SEN MOXALACTAM - IN CEFOTAXIME - IN CEFOPERAZONE - SEN CEFSULODIN - SEN COLISTIN SULFADIAZINE - SEN
000-81 DOE, JANE Taken:24-DEC-81 , 0500 Assn:81-12-24-07	URINE 1.0X10^3	ENTERIC E COLI	AMIKACIN - SEN GENTAMICIN - SEN TOBRAMYCIN - SEN TICARCILLIN - SEN MEZLOCILLIN - SEN PIPERACILLIN - SEN MOXALACTAM - SEN CEFOTAXIME - SEN CEFOPERAZONE - SEN CEFSULODIN - SEN COLISTIN - SEN SULFADIAZINE - SEN
000-81 DOE, JANE Taken:24-DEC-82 , 0500 Assn:82-12-24-13	SPUTUM 2.3X10^5	ENTERIC K PNEUMO	AMIKACIN - SEN GENTAMICIN - SEN TOBRAMYCIN - SEN TICARCILLIN - SEN MEZLOCILLIN - SEN PIPERACILLIN - SEN MOXALACTAM - SEN CEFOTAXIME - SEN CEFOPERAZONE - SEN CEFSULODIN - RES COLISTIN - SEN SULFADIAZINE - IN

Table 4. PATIENT X'S MICRO RECORD BY POST BURN DAY

POST BURN DAY	WARD	YRMN	DY	SOURCE NAME	SOUCE DESCRIPTION	ORGANISM NAME	Q1	Q2	AS
1	1	8208	15	BLOOD				0	
1	1	8208	14	URINE				0	
2	1	8208	16	BLOOD				0	
2	1	8208	16	SPUTUM		S VIRID	2	6	
2	1	8208	16	SPUTUM		N-HEM NO D	2	6	
2	1	8208	16	URINE				0	
2	1	8208	16	SWAB/RECTU		E COLI		0	*
2	1	8208	16	SWAB/RECTU		P STUARTII		0	
2	1	8208	35	CONTACT PL	L KNEE			0	
4	1	8208	18	URINE		P AERUG	1.2	4	*
4	1	8208	18	SWAB/RECTU		E COLI		0	
4	1	8208	18	SWAB/RECTU		P MIRAB		0	
4	1	8208	18	SWAB/RECTU		K PNEUMO		0	
5	1	8208	29	URINE				0	
6	1	8208	19	BLOOD				0	
6	1	8208	20	BLOOD				0	
6	1	8208	20	URINE		P AERUG	>	5	*
6	1	8208	20	CONTACT PL	R KNEE	P AERUG		0	*
6	1	8208	20	CONTACT PL	R KNEE	S EPI		0	
9	1	8208	23	BLOOD				0	
9	1	8208	23	URINE				0	
9	1	8208	23	SWAB/RECTU		E COLI		0	
9	1	8208	23	SWAB/RECTU		P MIRAB		0	
9	1	8208	23	CONTACT PL	R ARM			0	
9	1	8208	23	IV TIP	JUGULAR			0	
9	1	8208	23	SPUTUM		S VIRID	<1	4	
9	1	8208	23	SPUTUM		S AUREUS	<1	4	*
9	1	8208	23	SPUTUM		G D ENTERO	<1	4	
11	1	8208	25	URINE				0	
11	1	8208	25	SWAB/RECTU		C DIVERS		0	
11	1	8208	25	CONTACT PL	L KNEE 1-10 COLONIES	S EPI		0	*
12	1	8208	25	BLOOD				0	
13	1	8208	27	BLOOD				0	
13	1	8208	27	URINE				0	
14	1	8208	27	BLOOD				0	
14	1	8208	28	BLOOD				0	
15	1	8208	29	BLOOD				0	
15	1	8208	29	BLOOD				0	
16	1	8208	29	BLOOD				0	
16	1	8208	30	BLOOD-!! P		P AERUG		0	*
16	1	8208	30	SWAB/RECTU		E COLI		0	
16	1	8208	30	SWAB/RECTU		K PNEUMO		0	
16	1	8208	30	IV TIP	R SUBCLAVIAN LINE	C ALBICANS		0	
17	1	8208	31	BLOOD				0	
18	1	8209	01	BLOOD				0	
18	1	8209	01	SWAB/RECTU		E COLI		0	
18	1	8209	01	SWAB/RECTU		S AUREUS		0	
19	1	8209	01	BLOOD				0	
19	1	8209	01	URINE				0	
20	1	8209	03	BLOOD				0	
21	1	8209	03	URINE				0	
23	1	8209	03	BLOOD				0	

Table 4. PATIENT X'S MICRO RECORD BY POST BURN DAY

(Continued)

POST BURN DAY	WARD	YRMN	DY	SOURCE NAME	SOUCE DESCRIPTION	ORGANISM NAME	Q1	Q2	AS
23	1	8209	06	BLOOD				0	
23	1	8209	05	BLOOD				0	
23	1	8209	05	URINE				0	
23	1	8209	06	URINE				0	
23	1	8209	06	SWAB/RECTU		K PNEUMO		0	
23	1	8209	06	SWAB/RECTU		E COLI		0	
23	1	8209	06	SWAB/RECTU		P VULG B-3		0	*
23	1	8209	05	STOOL		E COLI		0	
23	1	8209	05	STOOL		K PNEUMO		0	
23	1	8209	05	STOOL		S AUREUS		0	*
25	1	8209	08	BLOOD				0	
25	1	8209	08	URINE		C RUGOSA	2	3	
25	1	8209	08	SWAB/RECTU		E COLI		0	
25	1	8209	08	SWAB/RECTU		C RUGOSA		0	
27	1	8209	10	BLOOD				0	
27	1	8209	10	URINE		C TROPICAL	7	3	
30	1	8209	13	SWAB/RECTU		E COLI		0	
30	1	8209	13	SWAB/RECTU		P MIRAB		0	
30	1	8209	13	SWAB/RECTU		K OXYTOCA		0	
30	1	8209	13	BLOOD				0	
30	1	8209	13	URINE				0	
31	1	8209	13	SPUTUM		S AUREUS	<1	4	*
31	1	8209	13	SPUTUM		N-HEM NO D	<1	4	
31	1	8209	13	SPUTUM		S VIRID	<1	4	
32	1	8209	15	BLOOD				0	
32	1	8209	15	URINE		P AERUG	1	4	*
32	1	8209	15	URINE		P STUARTII	1	4	
32	1	8209	15	SWAB/RECTU		E COLI		0	
32	1	8209	15	SWAB/RECTU		S AUREUS		0	*
32	1	8209	15	SWAB/RECTU		G D ENTERO		0	
32	1	8209	15	SWAB/RECTU		S VIRID		0	
34	1	8209	17	BLOOD				0	
34	1	8209	17	URINE		P STUARTII	4.2	4	
34	1	8209	17	SPUTUM		S AUREUS	2.2	7	*
34	1	8209	17	SPUTUM		S VIRID	2.2	7	
34	1	8209	17	SPUTUM		N-HEM NO D	2.2	7	
37	1	8209	20	URINE		P AERUG	7.5	6	*
37	1	8209	20	URINE		P STUARTII	7.5	6	
37	1	8209	20	SWAB/RECTU		E COLI		0	
37	1	8209	20	SWAB/RECTU		K PNEUMO		0	
37	1	8209	20	SWAB/RECTU		P MIRAB		0	
37	1	8209	20	SWAB/RECTU		G D ENTERO		0	
37	1	8209	20	SPUTUM		S AUREUS	3	6	*
37	1	8209	20	SPUTUM		S VIRID	3	6	
37	1	8209	20	BLOOD				0	
39	1	8209	22	SWAB/RECTU		E COLI		0	
39	1	8209	22	SWAB/RECTU		K PNEUMO		0	
39	1	8209	22	SWAB/RECTU		P MIRAB		0	
39	1	8209	22	SWAB/RECTU		G D ENTERO		0	
40	1	8209	23	BLOOD				0	
64	1	8210	16	PORCINE GR	LOT#S7145	P AERUG		0	*
76	1	8210	29	SWAB/MISC	EXUDATE UPPER R FOOT	P AERUG		0	*

Table 5

PATIENT X'S MICRO RECORD BY ORGANISM

ORGANISM NAME	SOURCE NAME	YRMN	DY	WARD	Q1	Q2	AS
C DIVERS	SWAB/RECTU	8208	25	1			
E COLI	SWAB/RECTU	8208	16	1			*
E COLI	SWAB/RECTU	8208	18	1			
E COLI	SWAB/RECTU	8208	23	1			
E COLI	SWAB/RECTU	8208	30	1			
E COLI	SWAB/RECTU	8209	01	1			
E COLI	STOOL	8209	05	1			
E COLI	SWAB/RECTU	8209	06	1			
E COLI	SWAB/RECTU	8209	08	1			
E COLI	SWAB/RECTU	8209	13	1			
E COLI	SWAB/RECTU	8209	15	1			
E COLI	SWAB/RECTU	8209	20	1			
E COLI	SWAB/RECTU	8209	22	1			
K PNEUMO	SWAB/RECTU	8208	18	1			
K PNEUMO	SWAB/RECTU	8208	30	1			
K PNEUMO	STOOL	8209	05	1			
K PNEUMO	SWAB/RECTU	8209	06	1			
K PNEUMO	SWAB/RECTU	8209	20	1			
K PNEUMO	SWAB/RECTU	8209	22	1			
K OXYTOCA	SWAB/RECTU	8209	13	1			
P MIRAB	SWAB/RECTU	8208	18	1			
P MIRAB	SWAB/RECTU	8208	23	1			
P MIRAB	SWAB/RECTU	8209	13	1			
P MIRAB	SWAB/RECTU	8209	20	1			
P MIRAB	SWAB/RECTU	8209	22	1			
P VULG B-3	SWAB/RECTU	8209	06	1			*
P STUARTII	SWAB/RECTU	8208	16	1			
P STUARTII	URINE	8209	15	1	1	4	
P STUARTII	URINE	8209	17	1	4.2	4	
P STUARTII	URINE	8209	20	1	7.5	6	
P AERUG	URINE	8208	18	1	1.2	4	*
P AERUG	URINE	8208	20	1	>	5	*
P AERUG	CONTACT PL	8208	20	1			*
P AERUG	BLOOD-((P	8208	30	1			*
P AERUG	URINE	8209	15	1	1	4	*
P AERUG	URINE	8209	20	1	7.5	6	*
P AERUG	PORCINE GR	8210	16	1			*
P AERUG	SWAB/MISC	8210	29	1			*
P AERUG	SWAB/MISC	8210	31	2			*
P AERUG	SWAB/MISC	8211	13	2			*
S VIRID	SPUTUM	8208	16	1	2	6	
S VIRID	SPUTUM	8208	23	1	<1	4	
S VIRID	SPUTUM	8209	13	1	<1	4	
S VIRID	SWAB/RECTU	8209	15	1			
S VIRID	SPUTUM	8209	17	1	2.2	7	
S VIRID	SPUTUM	8209	20	1	3	6	
N-HEM NO D	SPUTUM	8208	16	1	2	6	
N-HEM NO D	SPUTUM	8209	13	1	<1	4	
N-HEM NO D	SPUTUM	8209	17	1	2.2	7	
N-HEM NO D	PORCINE GR	8210	18	2			
G D ENTERO	SPUTUM	8208	23	1	<1	4	
G D ENTERO	SWAB/RECTU	8209	15	1			
G D ENTERO	SWAB/RECTU	8209	20	1			
G D ENTERO	SWAB/RECTU	8209	22	1			
S AUREUS	SPUTUM	8208	23	1	<1	4	*
S AUREUS	SWAB/RECTU	8209	01	1			
S AUREUS	STOOL	8209	05	1			*
S AUREUS	SPUTUM	8209	13	1	<1	4	*
S AUREUS	SWAB/RECTU	8209	15	1			*
S AUREUS	SPUTUM	8209	17	1	2.2	7	*
S AUREUS	SPUTUM	8209	20	1	3	6	*
S AUREUS	PORCINE GR	8210	18	2			*
S AUREUS	SWAB/MISC	8211	13	2			*
S EPI	CONTACT PL	8208	20	1			
S EPI	CONTACT PL	8208	25	1			*
S EPI	SWAB/MISC	8210	29	1			
S EPI	SWAB/MISC	8210	31	2			
C ALBICANS	IV TIP	8208	30	1			
C RUGOSA	URINE	8209	08	1	2	3	
C RUGOSA	SWAB/RECTU	8209	08	1			
C TROPICAL	URINE	8209	10	1	7	3	

Table 6. PATIENT X'S PS. SENSITIVITY RECORD

POST BURN DAY	SOURCE NAME	ORGANISM NAME	AM	GM	NN	TIC	MZ	PIP	MOX	CTX	CFB	CEFS	CL	SD
4	URINE	P AERUG	S	S	R	S	S	S	S	S	S	S	S	R
6	URINE	P AERUG	S	S	R	S	S	S	S	S	S	S	S	R
6	CONTACT PL	P AERUG	S	S	R	S	S	S	R	R	S	S	S	R
16	BLOOD-II P	P AERUG	S	S	R	S	S	S	S	S	S	S	S	R
32	URINE	P AERUG	R	R	R	S	S	S	S	S	S	S	S	R
37	URINE	P AERUG	S	S	R	S	S	S	S	S	S	S	S	R

Table 7. PATIENT X'S PS. ZONE RECORD

POST BURN DAY	SOURCE NAME	AM1	GM1	NN1	TIC1	MZ1	PIP1	MOX1	CTX1	CFB1	CL1	SD1
4	URINE	20.4	16.8	10.2	25.8	23.6	30.6	22.6	20.0	28.7	15.2	6.0
6	URINE	19.6	14.9	9.8	26.4	24.8	32.1	21.4	19.0	28.6	14.4	6.0
6	CONTACT PL	18.0	14.6	10.0	12.7	13.6	26.3	12.4	9.0	22.5	14.6	6.0
16	BLOOD-II P	22.4	16.8	8.6	27.4	24.6	31.2	23.0	20.0	28.6	15.2	6.0
32	URINE	13.4	11.5	6.0	24.3	22.2	30.1	19.2	17.2	26.8	15.0	6.0
37	URINE	14.4	13.6	6.0	27.0	24.0	33.8	23.8	19.0	30.2	16.0	6.0

Table 8

TOTAL SOURCE AND ISOLATES PROGRAM
BETWEEN 1-OCT-81 AND 30-SEP-82

DISTRIBUTION BY SOURCE

NAME	TOTAL SPECIMENS	Number with NO GROWTH	Number with ISOLATES	Percent of ISOLATES	Number of PATIENTS	Percent of PATIENTS
POSITIVE BLOOD	236	0	236	7.24%	71	32.87%
NEGATIVE BLOOD	1809	1809	0	0.00%	177	81.94%
POSITIVE ARD	17	0	17	0.52%	8	3.70%
NEGATIVE ARD	96	96	0	0.00%	17	7.87%
I.V. TIP	286	191	95	2.91%	84	38.89%
SPUTUM	1351	57	1294	39.68%	110	50.93%
ORAL SWAB	24	0	24	0.74%	16	7.41%
BIOPSY	303	143	160	4.91%	52	24.07%
CONTACT PLATE	511	179	332	10.18%	150	69.44%
SWABS	464	236	228	6.99%	111	51.39%
HOMOGRAFT	38	28	10	0.31%	2	0.93%
AUTOGRAFT	2	2	0	0.00%	1	0.46%
PORCINE GRAFT	384	196	188	5.77%	87	40.28%
URINE	1394	945	449	13.77%	163	75.46%
FOLEY TIP	45	23	22	0.67%	35	16.20%
STOOL	8	0	8	0.25%	7	3.24%
RECTAL SWAB	202	5	197	6.04%	41	18.98%
CSF	1	1	0	0.00%	1	0.46%
PLEURAL FLUID	6	5	1	0.03%	5	2.31%
TOTALS	7177	3916	3261	100.00%	216	100.00%

Table 8. Growth was observed in 3,261 (45%) of submitted specimens, The distribution of patients cultured by source shows the blood, upper respiratory system and the wound were the most common sources of specimens.

The organisms isolated and the number of patients who yielded that organism are presented in Table 9. More than 70 species were identified. The 10 most common organisms are listed in Table 10. Note that it is the practice of the Microbiology Section to report all species isolated. This practice results in flora "normal" to nonburn hosts being included in the summary report. The flora of the most common culture sources will be presented separately.

FLORA RECOVERED FROM RESPIRATORY SYSTEM IN BURNED PATIENTS

Specimens from the respiratory system included: sputum, bronchoscopy, pleural fluid, oral swabs and tracheoscopy samples. A total of 3,425 isolates were obtained from 118 patients. A total of 51 species were isolated. The ten most common isolates from the upper respiratory sources are presented in Table 11. Non-hemolytic streptococci were, as expected, the predominant flora isolated. In the absence of clinical signs of respiratory tract infection, these isolates represent normal flora. In addition, the streptococci as a group, with the exception of the enterococci, were not a significant part of the flora isolated in blood culture. Gram-negative bacilli were a significant part of the opportunistic colonizers observed. Pseudomonas aeruginosa, Klebsiella pneumoniae and Providencia stuartii were predominant and colonized approximately half of cultured patients. These three organisms plus Staphylococcus aureus and the enterococcus represent the clinically significant flora observed.

FLORA RECOVERED FROM BURN WOUNDS

Measurement of wound surfaces by swab technique showed 236 of 571 cultures (45%) to be negative. Among positive cultures, 48 patients of 89 cultured yielded Pseudomonas aeruginosa (54%). Staphylococcus aureus was found on 36 patient wounds (40.5%). Providencia stuartii was found on 25 patient wounds (28%). More than 10% of patients were found colonized by Escherichia coli, Klebsiella pneumoniae or Staphylococcus epidermidis.

Subsurface flora was measured in 433 burn wound biopsies. No growth was found in 52 specimens (33%). This is a continuance of low recoveries in previous reporting periods. The possibility that residual chlorhexidine gluconate from wound washing lowers recoveries is being investigated. Organisms found in more than 10% of patients are presented in Table 12. With the exception of Escherichia coli, the burn wound biopsy predominant species are very similar to the flora isolated in blood cultures (see below).

FLORA RECOVERED FROM URINARY TRACT OF BURNED PATIENTS

Specimens included as urinary tract sources were urine and Foley

Table 9. Distribution by Organism

Organism	No. Isolates	No. Patients Colonized	Organism	No. Isolates	No. Patients Colonized
Acinetobacter anitratus	36	15	Morganella morganii	13	7
Acinetobacter lwoffii	2	2	Neisseria sp.	21	10
Citrobacter freundii	29	16	Serratia marcescens	101	16
Citrobacter diversus	55	20	Serratia liquefaciens	11	5
Escherichia coli	299	72	Group A Streptococcus	11	5
Enterobacter cloacae	52	30	Group B Streptococcus	3	3
Enterobacter aerogenes	37	21	Beta Streptococcus not Group A, B, D	57	23
Enterobacter agglomerans	21	13	Streptococcus viridans	452	98
Enterobacter gergoviae	2	2	Non-hemolytic Streptococcus not Group D	250	91
Klebsiella pneumoniae	486	80	Group D Streptococcus not Enterococcus	32	23
Klebsiella oxytoca	50	22	Group D Enterococcus	274	58
Klebsiella ozaenae	13	9	Streptococcus pneumoniae	27	14
Pseudomonas aeruginosa	1116	97	Staphylococcus aureus	773	120
Pseudomonas fluorescens	17	10	Staphylococcus epidermidis	228	87
Pseudomonas putida	72	26	Micrococcus sp.	8	7
Pseudomonas cepacia	10	7	Corynebacterium sp.	2	2
Pseudomonas alcaligenes	4	4	Candida albicans	108	20
Pseudomonas maltophilia	13	3	Candida rugosa	203	46
Proteus mirabilis	203	51	Candida tropicalis	56	14
Proteus vulgaris	8	6	Yeast sp.	36	23
Proteus rettgeri	6	2	True fungi sp.	13	10
Providencia stuartii	899	81			
Aeromonas hydrophilia	3	2			
Alcaligenes faecalis	3	3			
Flavobacterium sp	5	5			
Hafnia alvei	25	4			
Haemophilus influenzae	3	1			
Haemophilus aphrophilus	1	1	Total isolates = 6398; total patients = 216		

Table 10. Ten Most Frequent Organisms in Total Flora

Organism	No. Patients Colonized	% Patients	No. Strains Isolated	% Total Isolates
Staphylococcus aureus	120	55.56	773	11.55
Streptococcus viridans	98	45.37	452	7.12
Pseudomonas aeruginosa	97	44.91	1116	17.58
Non-Group D non-hemolytic streptococcus sp.	91	42.13	250	3.94
Staphylococcus epidermidis	87	40.28	228	3.59
Providencia stuartii	81	37.50	899	14.16
Klebsiella pneumoniae	80	37.04	486	7.66
Escherichia coli	72	33.33	299	4.71
Group D enterococcus	58	26.85	274	4.32
Proteus mirabilis	51	23.61	203	3.20
			4980	77.83
Total patients cultured = 216				

Table 11. Ten Most Frequent Isolates from Respiratory System Flora

Organism	No. Patients Colonized	% Patients	No. Strains Isolated	% Resp. Isolates
Non-hemolytic streptococcus not Group D	91	77.12	198	5.78
Streptococcus viridans	88	74.58	418	12.20
Staphylococcus aureus	79	66.95	502	14.66
Pseudomonas aeruginosa	67	56.78	709	20.70
Klebsiella pneumoniae	64	54.24	273	7.97
Providencia stuartii	41	34.75	381	11.12
Staphylococcus epidermidis	40	33.90	84	2.45
Group D enterococcus	37	31.36	174	5.08
Escherichia coli	27	22.88	56	1.64
Beta hemolytic streptococcus sp. not Group A, B or D	23	19.49	52	1.52
			2847	83.24
Total patients cultured = 118				

Table 12. Organisms Found in More than Ten Percent of Burned Patient Biopsies

Organism	No. Patients Colonized	% Patients	No. Strains Isolated	% Total Isolates
Pseudomonas aeruginosa	28	53.9	67	23.1
Providencia stuartii	22	42.3	59	20.3
Staphylococcus aureus	10	19.2	13	4.5
Escherichia coli	9	17.3	10	3.5
Candida rugosa	8	15.4	25	8.6
Klebsiella pneumoniae	6	11.5	6	2.1

Total patients biopsied = 52; total strains isolated = 290

catheter tips. A total of 1,631 specimens were received from 166 patients. A total of 33 species were recovered. The 10 most common species are presented in Table 13. These 10 species represent more than 85% of the total organisms isolated. Providencia stuartii was the most frequent single species, being found in 35% of colonized patients. Candida species, however, represent the most significant group of organisms observed, with 36 of the 88 colonized patients positive (41%).

A summary of organisms recovered at more than 100,000 organisms/ml of urine is presented in Table 14. The 10 organisms listed represent 91% of all urine isolates with greater than 10^5 organisms/ml.

FLORA RECOVERED IN BLOOD CULTURE

A technical change in blood culture methods was introduced during this reporting period. The BACTEC 460 (Johnston Laboratories) automated blood culture system was used. The system uses radioactive substrates to measure the presence of metabolizing organisms in blood samples. The system cultures blood under strict anaerobic as well as aerobic conditions. As previously reported using manual methods, no anaerobic flora was observed in 2,180 blood cultures collected from 179 patients. The principal organisms isolated during 1982 are presented in Table 15. More than 80% of isolates and patients were positive for seven species. For the first time in several years, Providencia stuartii was the most common organism in blood. There were 28 cases in 26 patients with Providencia stuartii. A bacteremia (or fungemia) case is arbitrarily defined as the occurrence of a species in a blood specimen. If the patient has subsequent positive blood cultures with the same organism within 10 days of a previous isolation, the case continues. The definition is believed to cover our most common period of specific antibiotic treatment. Use of this definition did not result in two cases of bacteremia for the same organism occurring within any 30-day period in a patient.

There were 22 species isolated from a total of 171 bacteremia cases. The case incidence of bacteremia per calendar month is presented in Figure 1. The bacteremia rates as defined by (cases/mean monthly census) X 100 are presented in Figure 2. The case incidence by calendar month for the principal organisms isolated in blood is presented in Figures 3 to 9.

There were significant changes in the flora found in burned patient blood cultures during this reporting period. A comparison of bacteremic patients in 1981 and 1982 is presented in Table 16. As mentioned above, Providencia stuartii has reappeared as a significant blood stream inhabitant. Two other species have also become obvious. The Group D enterococcus and an unusual Candida species, Candida rugosa, have occurred in epidemic proportion. There was also an increase in frequency of Staphylococcus aureus bacteremia. The incidence of two important burned patient opportunistic pathogens, Pseudomonas aeruginosa and Klebsiella pneumoniae, did not change from the previous year. Staphylococcus epidermidis occurred at the same rate as 1981. In 1981, 17 isolates were observed once in 17 patient specimens. In 1982, 15 isolates were observed once in 15 patient

Table 13. Ten Most Frequent Organisms from Urinary Specimens

Organism	No. Patients Colonized	% Patients	No. Strains Isolated	% Urin. Isolates
Providencia stuartii	31	35.2	134	21.2
Candida albicans	9	10.2	82	13.0
Candida rugosa	21	23.9	73	11.6
Escherichia coli	17	19.3	63	10.0
Pseudomonas aeruginosa	21	23.9	58	9.2
Klebsiella pneumoniae	22	25.0	55	8.7
Proteus mirabilis	10	11.4	27	4.3
Candida tropicalis	5	5.7	26	4.1
Non-hemolytic streptococcus not Group D	18	20.5	22	3.5
Group D enterococcus	10	11.4	19	3.0

Total patients tested = 166; total patients positive = 88

Total cultures = 1631; total isolates = 630

Table 14. Ten Most Frequent Organisms from Urinary Specimens with $\geq 10^5$ CFU

Organism	No. Patients Colonized	% Patients	No. Strains Isolated	% Urin. Isolates
Providencia stuartii	12	34.3	60	20.9
Candida albicans	5	14.3	44	15.4
Escherichia coli	11	31.4	41	14.3
Pseudomonas aeruginosa	10	28.6	25	8.7
Klebsiella pneumoniae	9	25.7	20	6.9
Candida rugosa	9	25.3	20	6.9
Candida tropicalis	4	11.4	16	5.6
Proteus mirabilis	6	17.1	16	5.6
Citrobacter freundii	2	5.7	12	4.2
Staphylococcus epidermidis	2	5.7	5	1.7

Total isolates = 286; total patients = 35

Table 15. Principal Organisms Found in Blood Cultures from Burned Patients

Organism	No. Strains Isolated	% Total Isolates	No. Cases	% Cases	No. Patients
Providencia stuartii	55	20.0	28	16.4	26
Staphylococcus aureus	56	20.4	24	14.0	22
Pseudomonas aeruginosa	30	10.9	21	12.3	20
Candida rugosa	38	13.8	19	11.1	16
Group D enterococcus	29	10.5	19	11.1	15
Staphylococcus epidermidis	15	5.5	15	8.8	15
Klebsiella pneumoniae	15	5.5	11	6.4	10
	238	86.6	171	80.1	

Table 16. Patients with Positive Blood Cultures

Organism	1981	1982	1981 vs. 1982
Providencia stuartii	11	26	$P < 0.02$
Staphylococcus aureus	9	22	$P < 0.02$
Pseudomonas aeruginosa	19	20	$P = 0.90$*
Candida rugosa	0	16	$P < 0.001$
Enterococcus	2	15	$P < 0.002$
Staphylococcus epidermidis	17	15	$P = 0.68$*
Klebsiella pneumoniae	6	10	$P = 0.32$*
Total Candida species	2	25	$P < 0.001$

1981, 176 patients sampled; 1982, 179 patients sampled

* N.S.

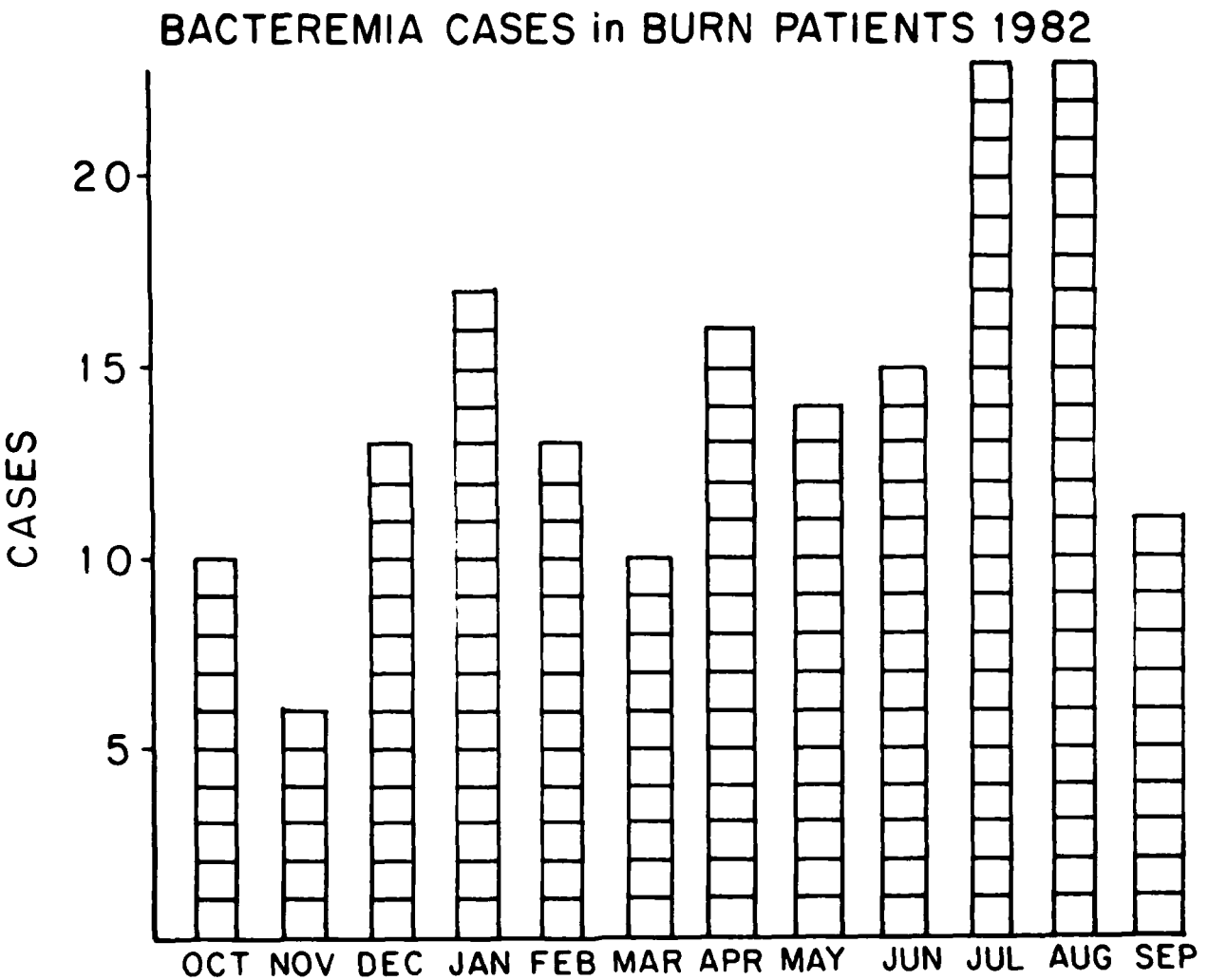

Figure 1. Cases of bacteremia and/or candidemia by month FY 82.

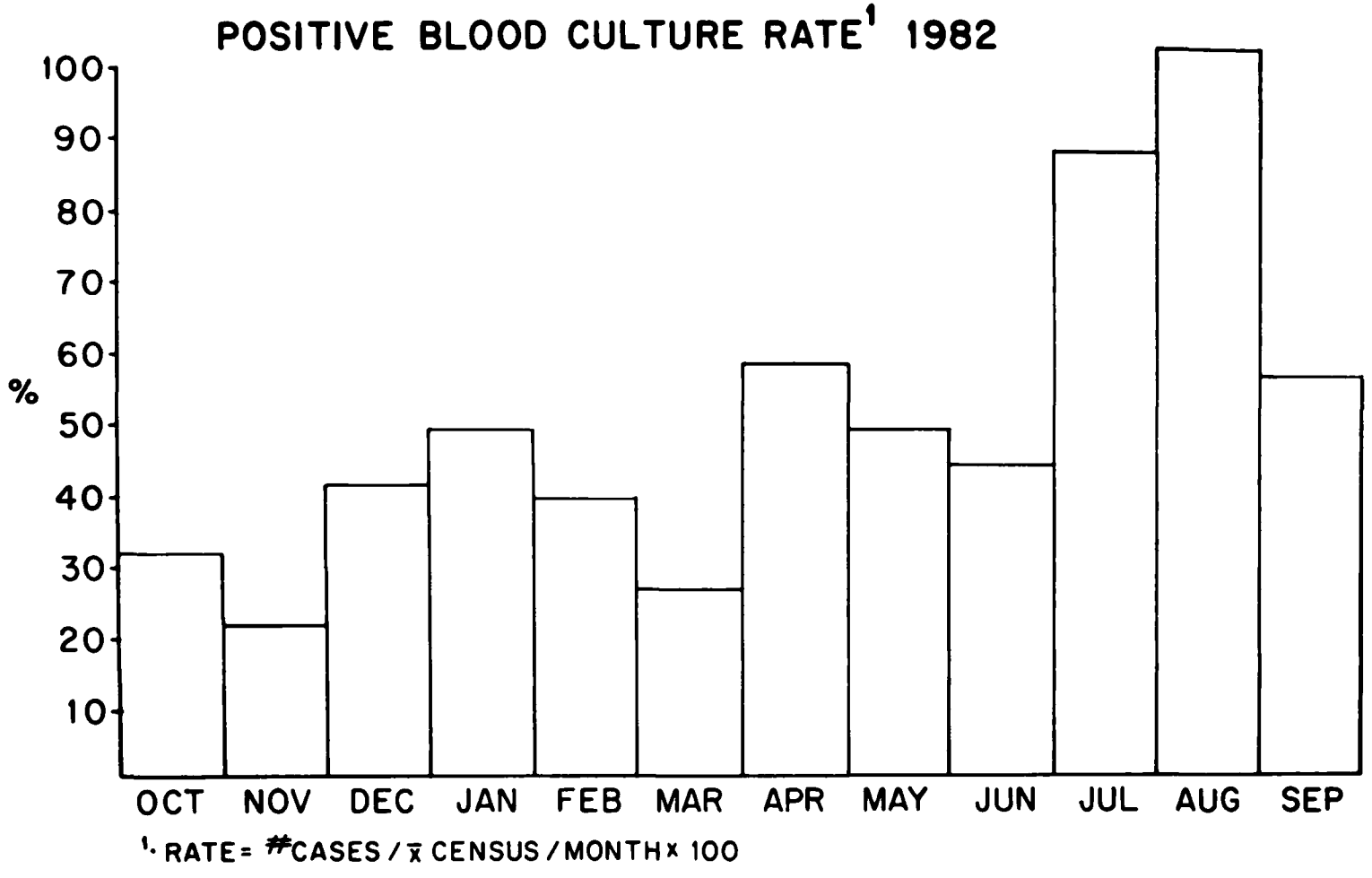

Figure 2. Rate of positive blood cultures FY 82.

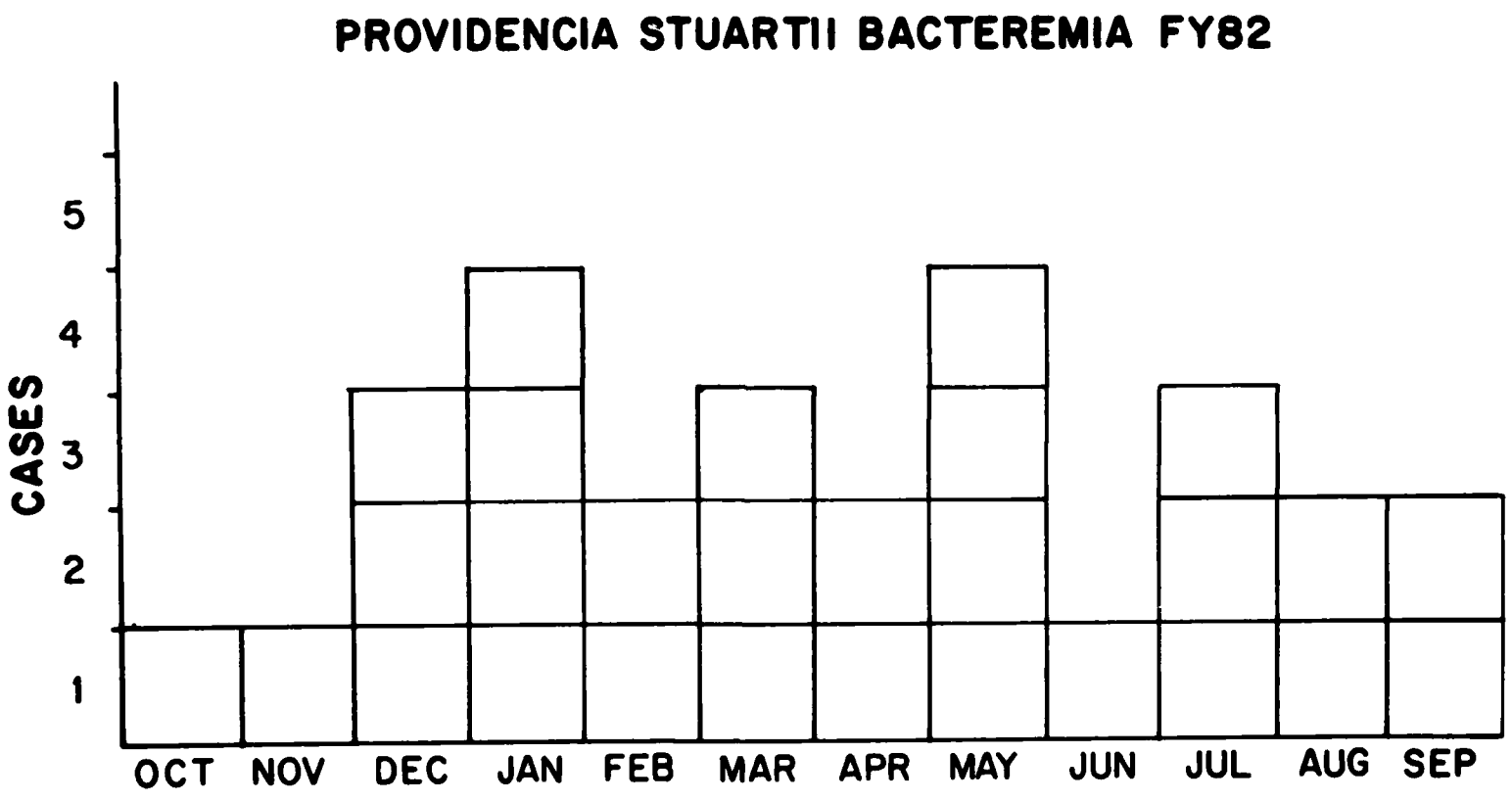

Figure 3. Incidence of Providencia stuartii bacteremia cases FY 82.

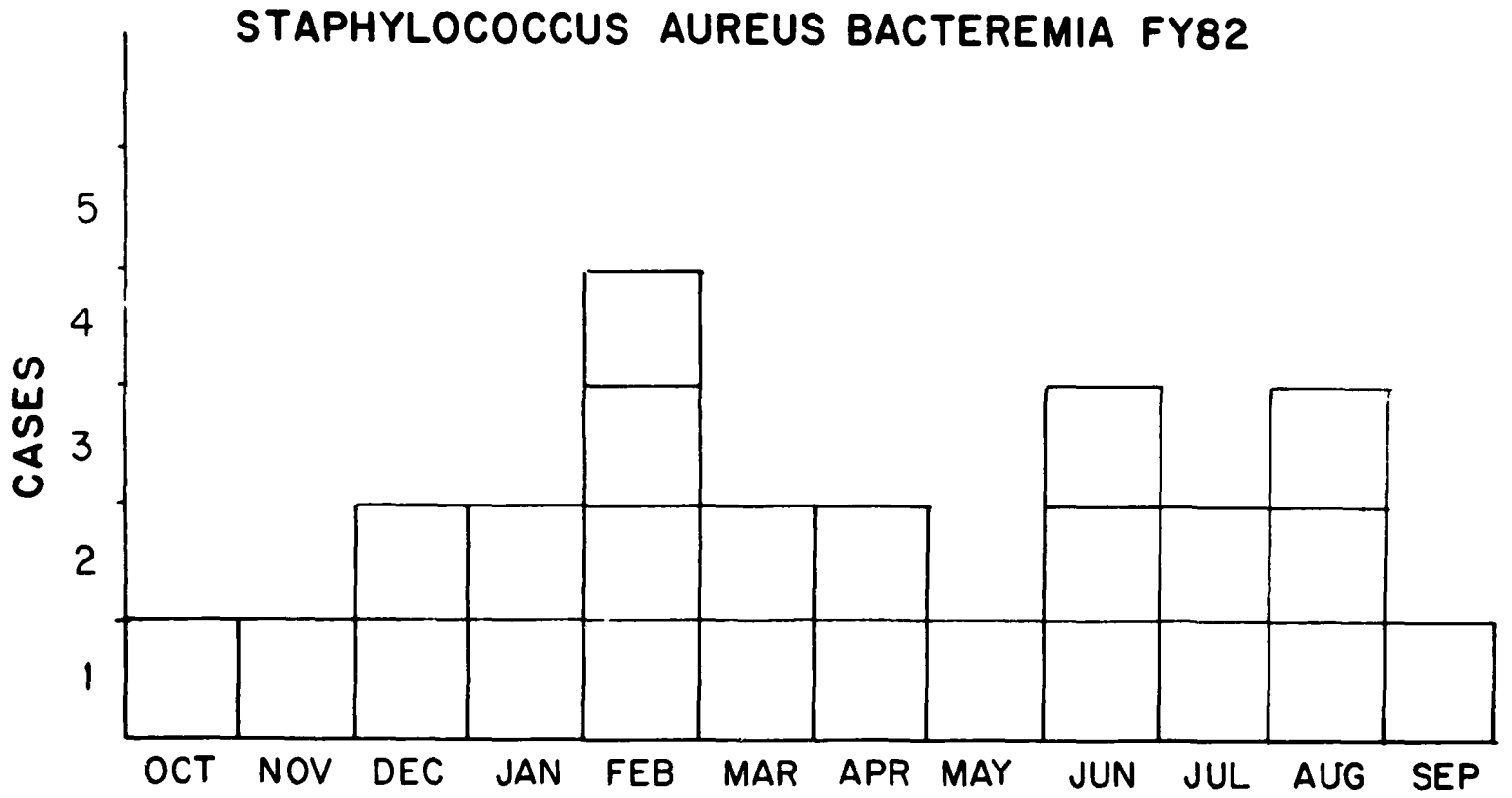

Figure 4. Incidence of Staphylococcus aureus bacteremia cases FY 82.

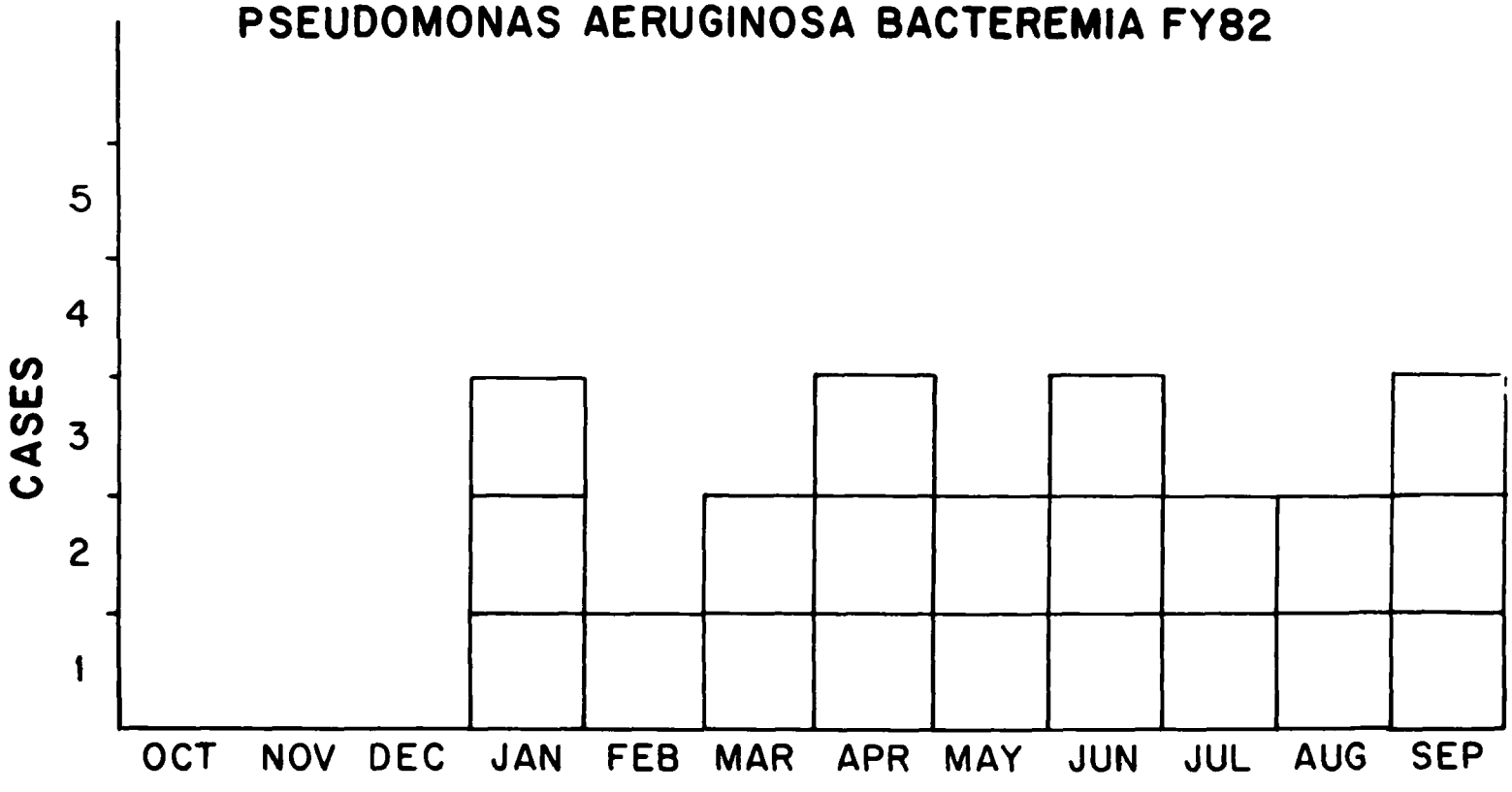

Figure 5. Incidence of Pseudomonas aeruginosa bacteremia cases FY 82.

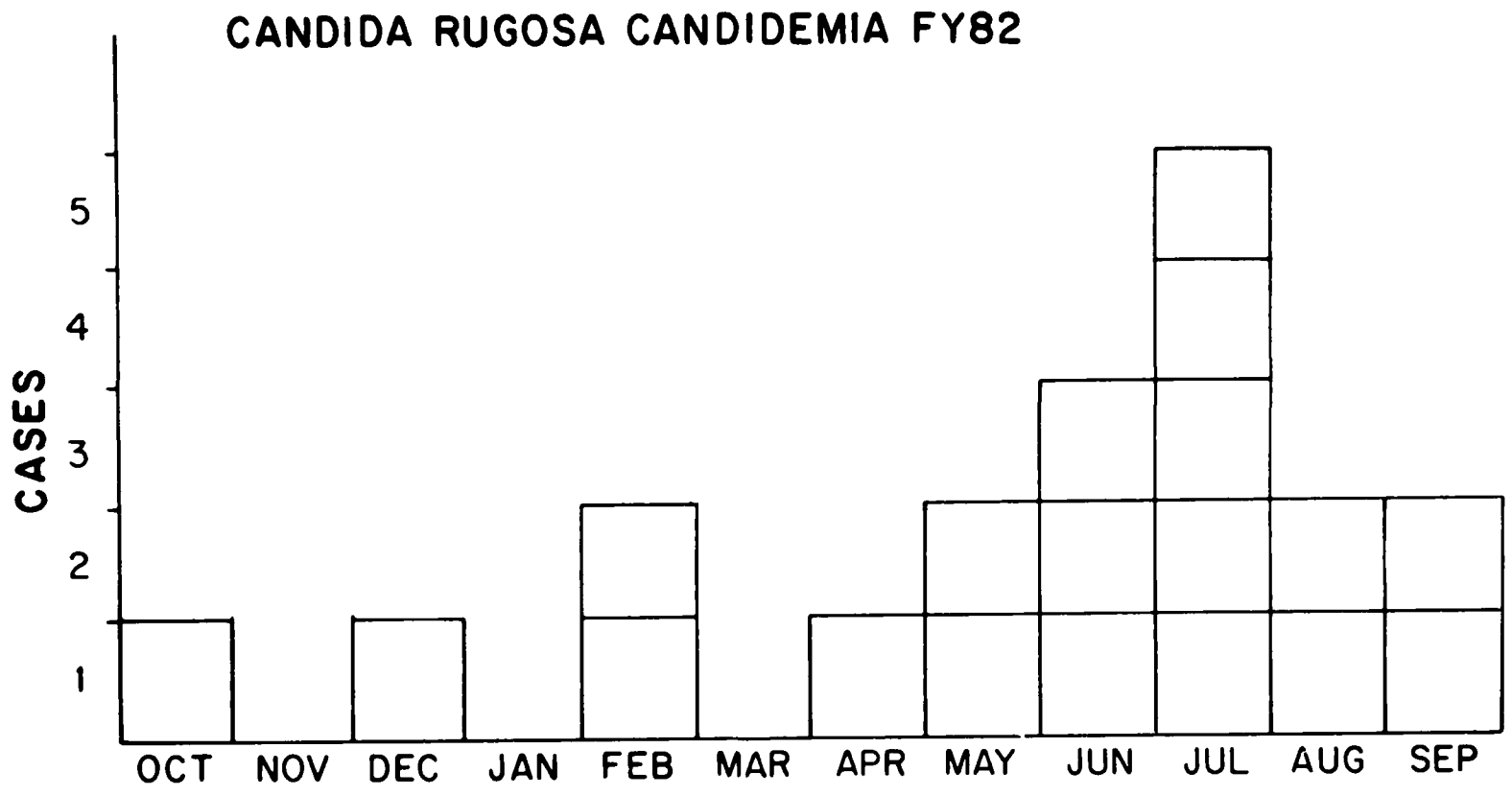

Figure 6. Incidence of Candida rugosa candidemia cases FY 82.

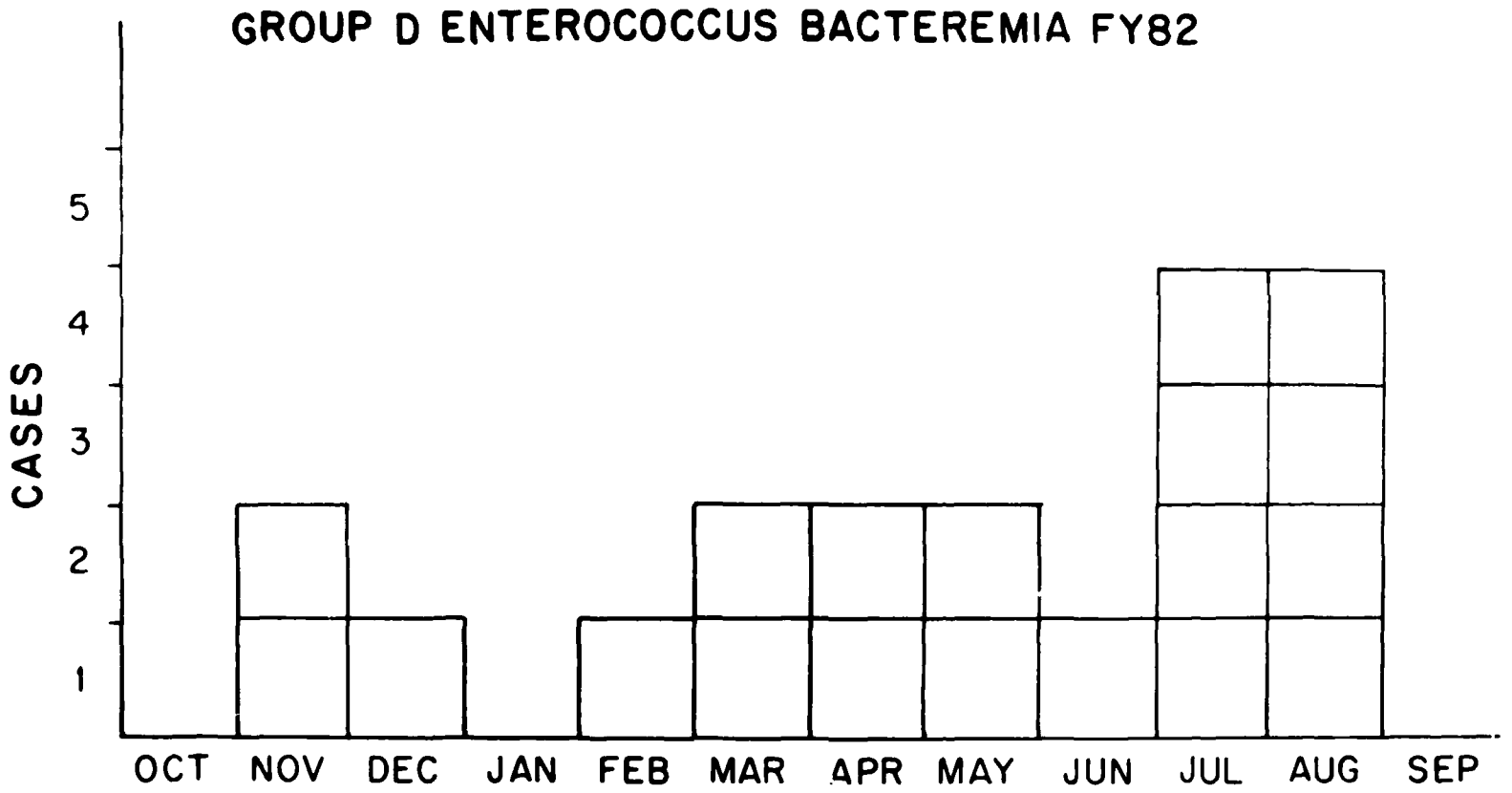

Figure 7. Incidence of Group D Enterococcus bacteremia cases FY 82.

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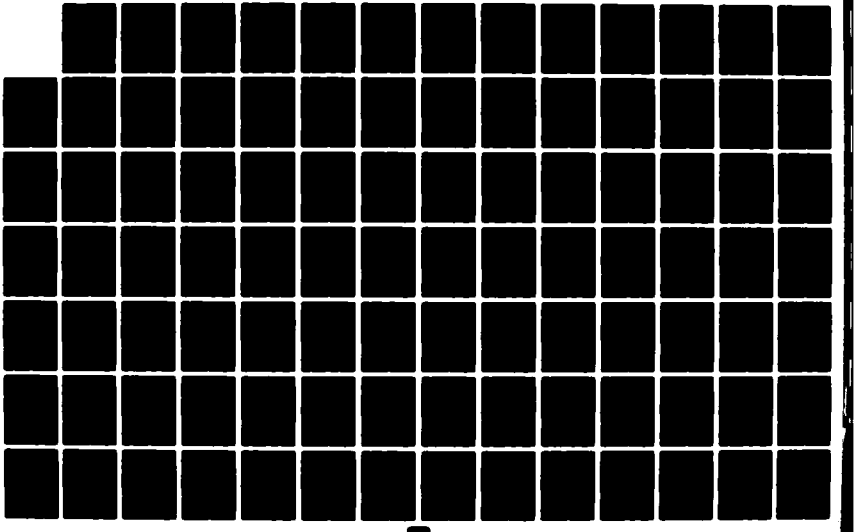

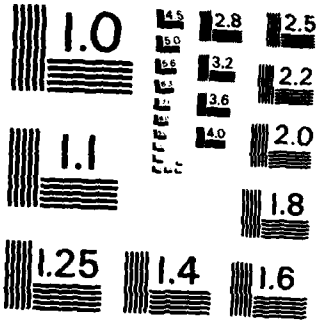

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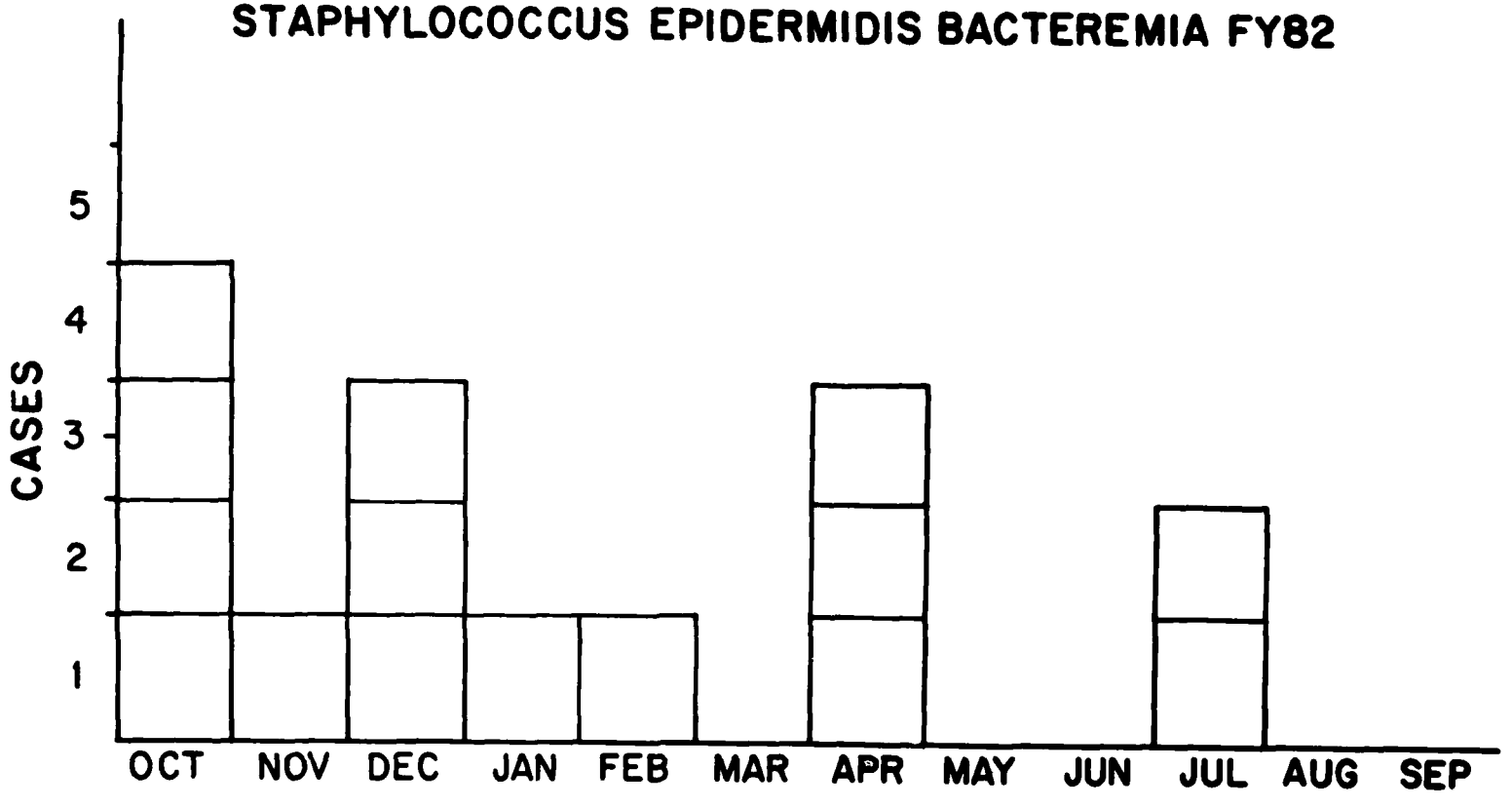


Figure 8. Incidence of Staphylococcus epidermidis bacteremia cases FY 82.

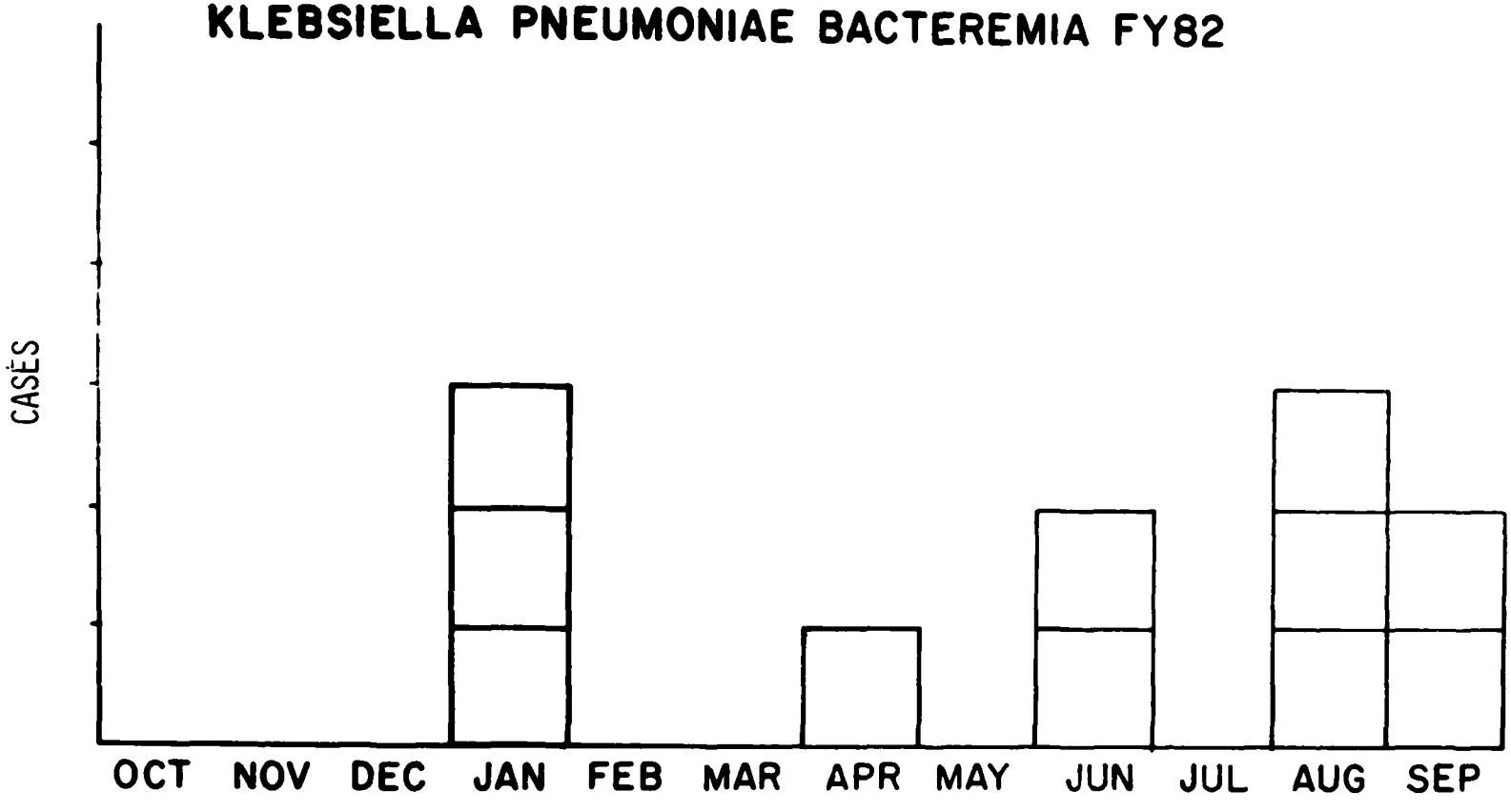


Figure 9. Incidence of Klebsiella pneumoniae bacteremia cases FY 82.

specimens. The similarity of isolation of this probable contaminant reflects on the equality of sampling technique between 1981 and 1982.

SUMMARY OF ANTIBIOTIC SENSITIVITY SURVEILLANCE

As reported in a previous section, antibiotic sensitivity testing has been markedly increased. During this reporting period, 2,178 isolates were examined for *in vitro* sensitivity. This compares to 216 isolates examined during the previous reporting period.

This summary will review the results for the organism types most frequently observed in blood cultures. Data will be presented by species collected from all sources. A separate comparison of blood stream isolates to total isolates will be presented.

PROVIDENCIA STUARTII SENSITIVITIES

A total of 357 isolates of *Providencia stuartii* were examined. The source distribution of the *tested* strains is presented in Table 17. The sensitivity percentages are presented in Table 18. The frequency distributions are presented in Figure 10a-f. As can be seen, the third generation cephalosporins (moxalactam, cefotaxime and cefoperazone) were the most active drugs against these isolates. Examination of strains collected during the past *Providencia* epidemic (1970-1976) shows the present strains to have distinctly different sensitivity patterns.

A total of 46 blood culture isolates were examined. The sensitivity percentages are presented in Table 19. Chi square comparisons of antibiotic sensitivity between blood and all other sources indicate there is no significant difference. This fact supports the concept that a single population of *Providencia stuartii* strains exists in our burn environment and that cross contamination is responsible for the consistency.

STAPHYLOCOCCUS AUREUS SENSITIVITIES

A total of 503 isolates of *Staphylococcus aureus* were examined. The source distribution of *tested* strains is presented in Table 20. The sensitivity percentages are presented in Table 21. Frequency distributions are in Figure 11a-f. The very high incidence of antibiotic sensitivity has continued. The weakest antibiotic tested was moxalactam which was extensively used early in the reporting period. Methicillin resistant staphylococci were less than 2% of the sample. No methicillin sensitive staphylococci were isolated in blood culture. This fact probably reflects the absence of use of penicillinase resistant penicillins for more than 5 years. A 20-year review of the frequency of methicillin resistance at this Institute is presented in Figure 12.

A total of 48 blood culture isolates were examined. The sensitivity percentages are presented in Table 22. No significant difference in sensitivity exists between blood isolates and isolates from all other sources, and there is no evidence of endemic resistant strains. The possibility of

Table 17. Sources of Providencia stuartii Tested for Antibiotic Sensitivity

Source	Number of Isolates
Sputum	135
Blood	51
Urine	47
Biopsy	41
Contact plate	33
Swabs	25
I.V. catheters	21
Porcine graft	3
Foley tip	1
Total	357

Table 18. Providencia stuartii Total Isolates Sensitivity

Antibiotic	Resistant %	Resistant No.	Intermediate %	Intermediate No.	Sensitive %	Sensitive No.	Total Tested
Amikacin	10.64	38	8.40	30	80.95	289	357
Gentamicin	98.31	350	0.56	2	1.12	4	356
Tobramycin	96.91	341	1.97	7	1.12	4	356
Ticarcillin	98.75	317	0.31	1	0.93	3	321
Mezlocillin	12.38	38	49.19	151	38.44	118	307
Piperacillin	11.64	39	50.45	169	37.91	127	335
Moxalactam	0.30	1	1.79	6	97.91	328	335
Cefotaxime	0.30	1	3.58	12	96.12	322	335
Cefoperazone	0.00	0	7.46	25	92.54	310	335
Cefsulodin	90.95	201	0.00	0	9.05	20	221
Colistin	98.60	351	0.56	2	0.84	3	356
Sulfadiazine	98.80	330	0.00	0	1.20	4	334

Table 19. Sensitivity of Blood Isolates of *Providencia stuartii*

Antibiotic	Resistant %	Resistant No.	Intermediate %	Intermediate No.	Sensitive %	Sensitive No.	Total Tested
Amikacin	17.39	8	4.35	2	78.26	36	46
Gentamicin	100.00	46	0.00	0	0.00	0	46
Tobramycin	97.83	45	2.17	1	0.00	0	46
Ticarcillin	100.00	22	0.00	0	0.00	0	22
Mezlocillin	34.78	8	30.43	7	34.78	8	23
Piperacillin	20.83	5	41.67	10	37.50	9	24
Moxalactam	0.00	0	0.00	0	100.00	24	24
Cefotaxime	0.00	0	0.00	0	100.00	24	24
Cefoperazone	0.00	0	16.67	4	83.33	20	24
Cefsulodin	90.48	19	0.00	0	9.52	2	21
Colistin	100.00	46	0.00	0	0.00	0	46
Sulfadiazine	100.00	24	0.00	0	0.00	0	24

Table 20. Sources of Staphylococcus aureus Tested for Antibiotic Sensitivity

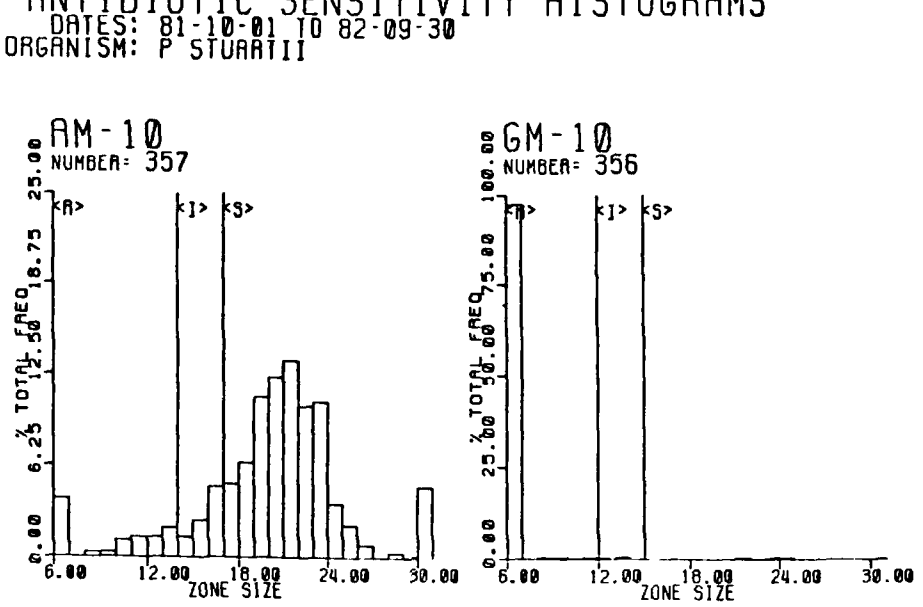
Source	No. of Isolates
Sputum	362
Blood	48
Swabs	36
Contact plates	33
Biopsy	8
I.V. catheters	6
Porcine graft	5
Urine	3
Stool	1
Foley tip	1
Total	503

Table 21. Staphylococcus aureus Total Isolates Sensitivity

Antibiotic	Resistant %	Resistant No.	Intermediate %	Intermediate No.	Sensitive %	Sensitive No.	Total Tested
Amikacin	0.83	4	0.41	2	98.77	481	487
Gentamicin	3.58	18	0.80	4	95.63	481	503
Tobramycin	3.19	16	1.79	9	95.02	477	502
Ticarcillin	0.00	0	0.91	4	99.09	434	438
Mezlocillin	0.69	3	4.37	19	94.94	413	435
Piperacillin	1.44	7	12.99	63	85.57	415	485
Moxalactam	4.11	20	88.09	429	7.80	38	487
Cefotaxime	0.82	4	10.27	50	88.91	433	487
Sulfadiazine	9.32	40	4.90	21	85.78	368	429
Methicillin	1.79	9	1.20	6	99.38	484	487
Cephalothin	0.21	1	0.41	2	99.38	484	487
Vancomycin	0.00	0	0.00	0	100.00	503	503

Table 22. Sensitivity of Blood Isolates of Staphylococcus aureus

Antibiotic	Resistant %	Resistant No.	Intermediate %	Intermediate No.	Sensitive %	Sensitive No.	Total Tested
Amikacin	0.00	0	0.00	0	100.00	0	32
Gentamicin	2.08	1	0.00	0	97.92	47	48
Tobramycin	2.08	1	0.00	0	97.92	47	48
Ticarcillin	0.00	0	0.00	0	100.00	32	32
Mezlocillin	0.00	0	0.00	0	100.00	27	31
Piperacillin	3.12	1	18.75	6	78.12	25	32
Moxalactam	0.00	0	87.50	28	12.50	4	32
Cefotaxime	0.00	0	6.25	2	93.75	30	32
Sulfadiazine	4.76	1	9.52	2	85.71	18	21
Methicillin	0.00	0	0.00	0	100.00	48	48
Cephalothin	0.00	0	0.00	0	100.00	32	32
Vancomycin	0.00	0	0.00	0	100.00	48	48



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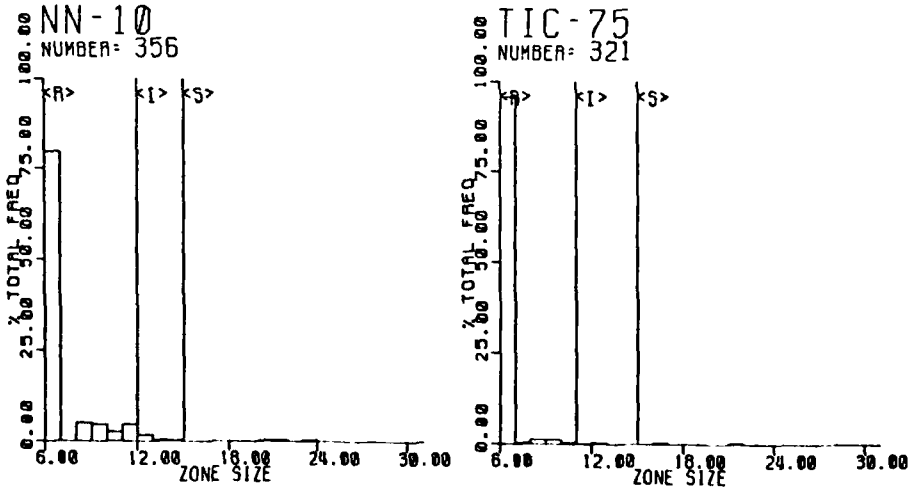
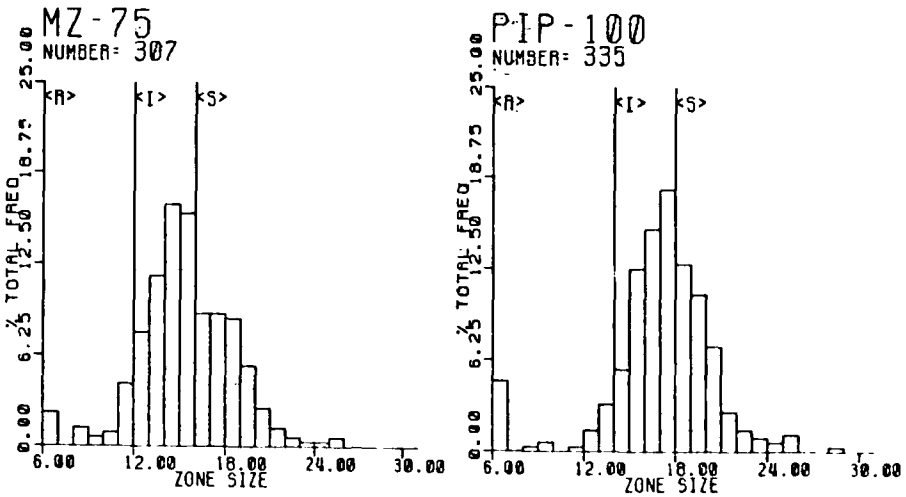


(b)

Figure 10. Antibiotics in (a): AM = amikacin (30 µg disc), GM = gentamicin (10 µg disc); antibiotics in (b): NN = tobramycin (10 µg disc), TIC = ticarcillin 75 µg disc).

ORGANISM: P STUARTII



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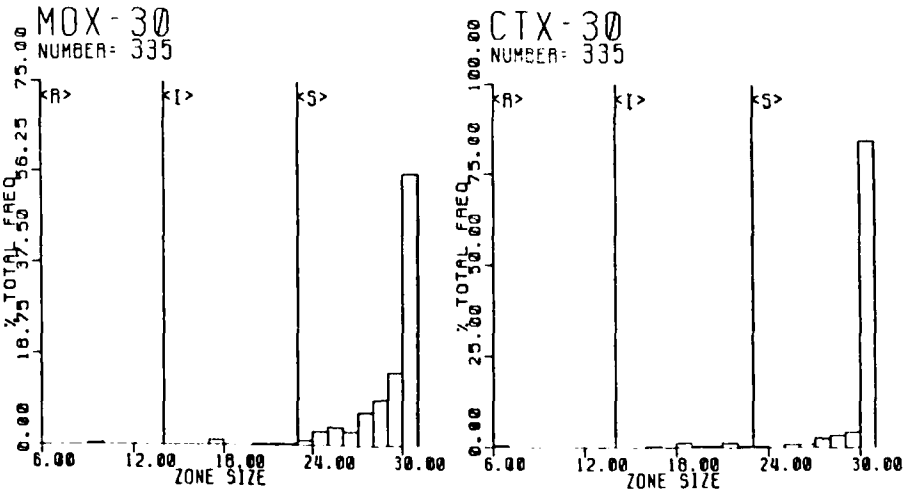
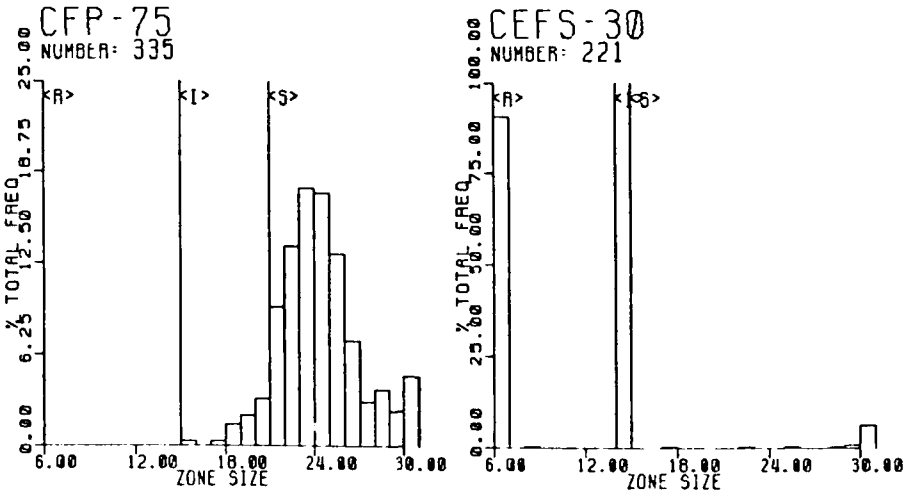
ORGANISM: P STUARTII



(d)

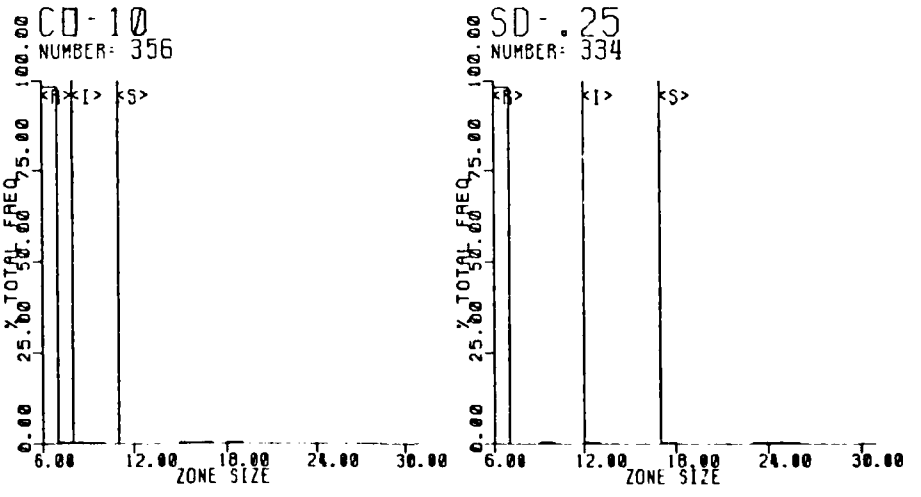
Figure 10. Antibiotics in (c): MZ = mezlocillin (75 µg disc), PIP = piperacillin (100 µg disc); antibiotics in (d): MOX = moxalactam (30 µg disc), CTX = cefotaxime (30 µg disc).

ORGANISM: P STUARTII



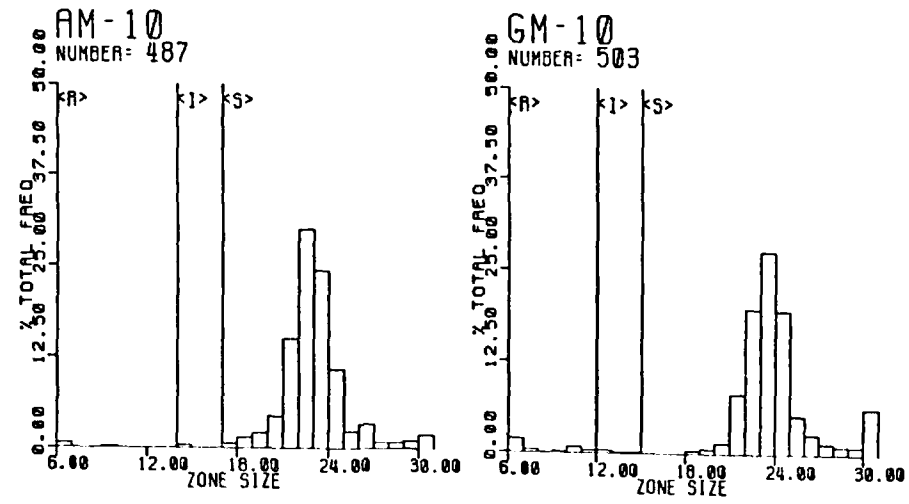
(e)

ORGANISM: P STUARTII



(f)

Figure 10. Antibiotics in (e): CFP = cefoperazone (75 µg disc), CEFS = cefsulodin (30 µg disc); antibiotics in (f): CO = colistin (10 µg disc), SD = sulfadiazine (250 µg disc).



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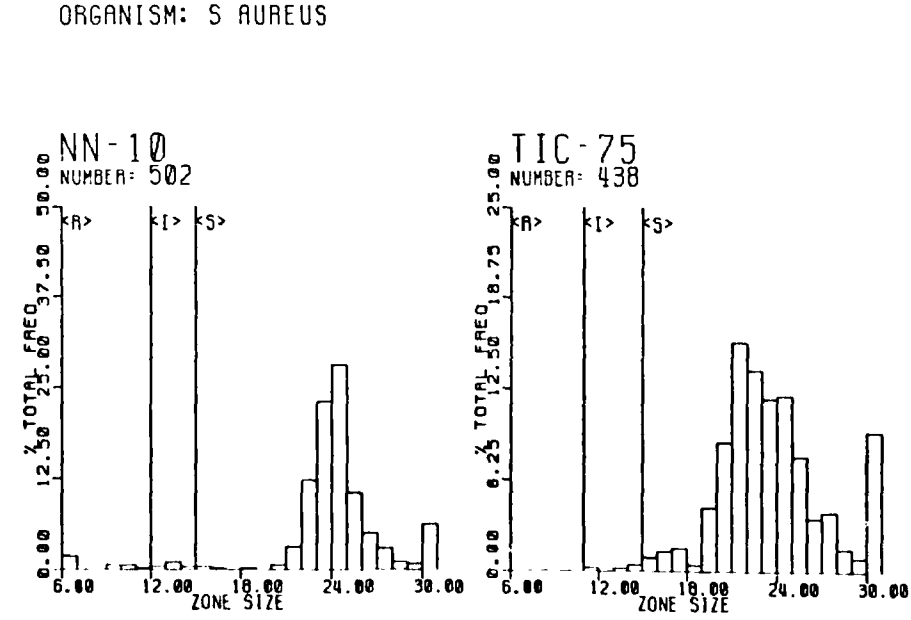
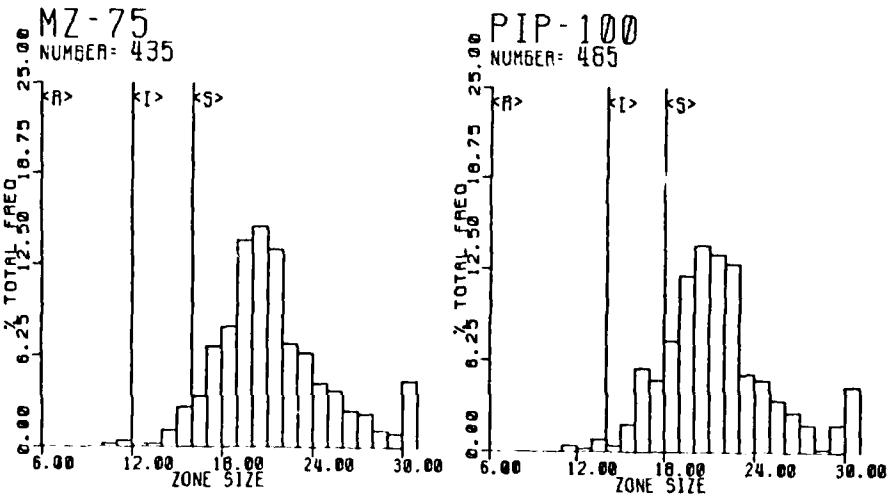


(b)

Figure 11. Antibiotics in (a): AM = amikacin (30 µg disc), GM = gentamicin (10 µg disc); antibiotics in (b): NN = tobramycin (10 µg disc), TIC = ticarcillin (75 µg disc).

ORGANISM: S AUREUS



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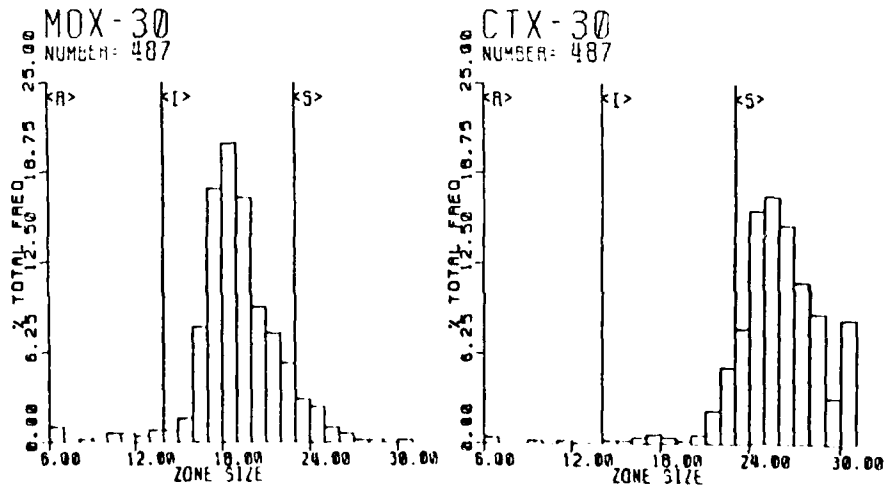
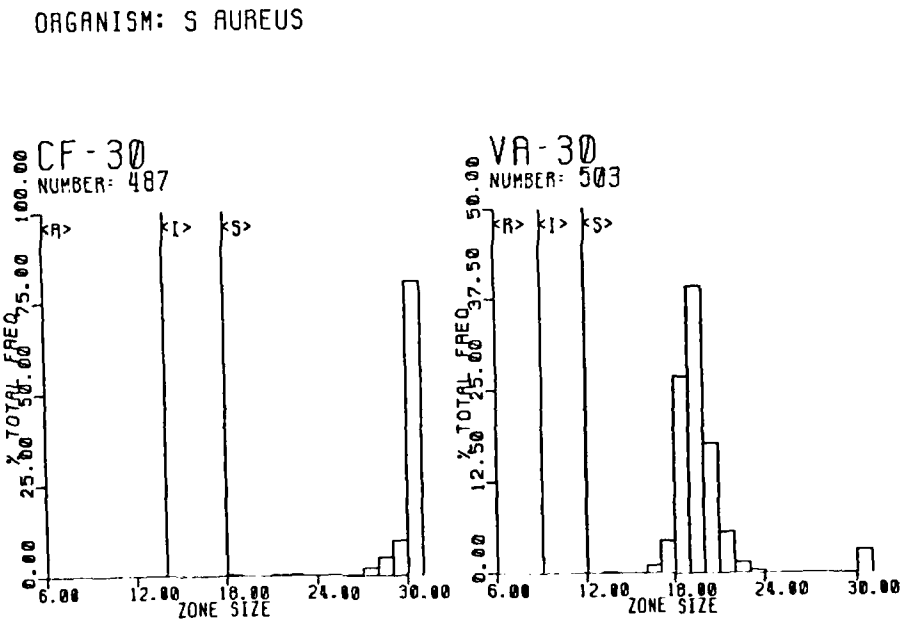
ORGANISM: S AUREUS



(d)

Figure 11. Antibiotics in (c): MZ = mezlocillin (75 µg disc), PIP = piperacillin (10 µg disc); antibiotics in (d): MOX = moxalactam (30 µg disc), CTX = cefotaxime (30 µg disc).



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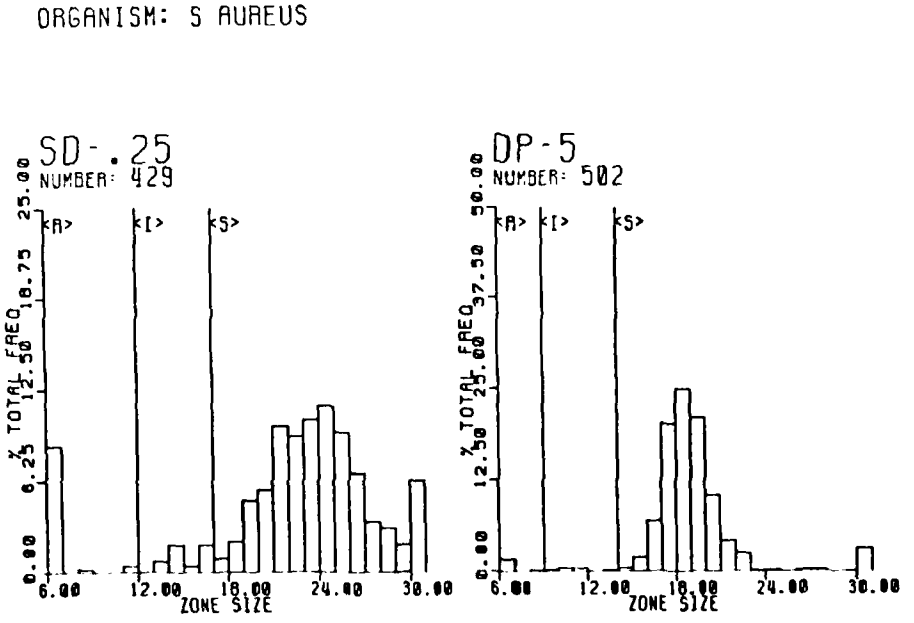

(f)

Figure 11. Antibiotics in (e): CF = cephalothin (30 µg disc), VA = vancomycin (30 µg disc); antibiotics in (f): SD = sulfadiazine (250 µg disc), DP = methicillin 5 µg).

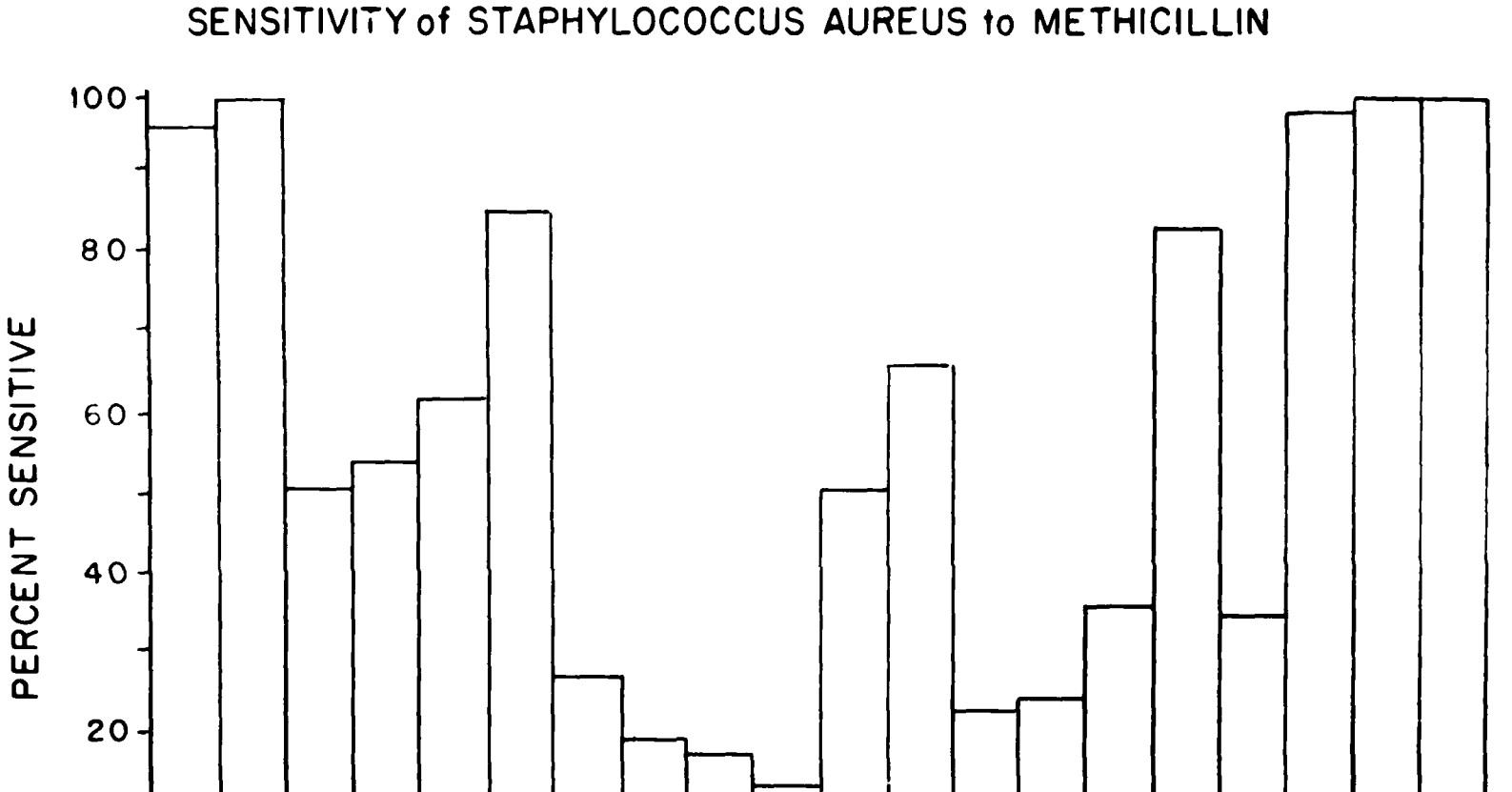

Figure 12. Incidence of methicillin resistant Staphylococcus aureus at the US Army Institute of Surgical Research.

an endemic sensitive strain does exist. This possibility is being investigated by phage typing.

PSEUDOMONAS AERUGINOSA SENSITIVITIES

A total of 804 isolates of Pseudomonas aeruginosa were examined. The source distribution of tested strains is presented in Table 23. The sensitivity percentages are presented in Table 24. Pseudomonas aeruginosa sensitivities were examined during the last reporting period (Annual Report, FY 81, pp 104-126). Frequency distribution for FY 82 results are presented with the corresponding frequency distribution for last year's data in Figures 13 through 24. The resistance frequency for tested antibiotics between years was compared by chi square analysis. Data are presented in Table 25. Marked changes were noted in antibiotic resistance during this reporting period. The extensive use of moxalactam during the reporting period was the only consistent change in chemotherapy. The aminoglycoside antibiotic testing all showed significant improvements in sensitivity. With the exception of cefotaxime, all tested beta-lactam antibiotics significantly lost sensitivity. Sulfonamide sensitivity significantly improved. Colistin sensitivity data are essentially identical between 1981 and 1982.

Blood isolate sensitivities are presented in Table 26. Chi square analysis of these data in comparison to total isolates showed that no difference in resistance existed between the two samples with the exception of sulfadiazine. Blood isolates were more resistant to sulfadiazine ($P < .04$).

Serologic epidemiologic data are presented in a separate section of the Institute's Annual Report.

GROUP D ENTEROCOCCUS SENSITIVITIES

A total of 68 isolates were examined. The source distribution is presented in Table 27. Sensitivity percentages are presented in Table 28. As can be seen, these strains show a high frequency of resistance to the third generation cephalosporins. This fact may explain the increased frequency of enterococci during the time when moxalactam was introduced to our clinical environment. Blood isolates examined ($n = 20$) show no significant difference in sensitivity from the total sample. Histogram data are not presented.

STAPHYLOCOCCUS EPIDERMIDIS SENSITIVITIES

A total of 27 isolates from 24 patients were examined. The source distribution is presented in Table 29. Antibiotic sensitivity percentages are presented in Table 30. As with Staphylococcus aureus, all strains were sensitive to vancomycin. The majority of strains isolated were blood isolates and therefore were not distinguishable within the sample. Histogram data are not presented.

Table 23. Sources of Pseudomonas aeruginosa Tested for Antibiotic Sensitivity

Source	Number of Isolates
Sputum	516
Swabs	79
Contact plates	69
Biopsy	55
Urine	38
Blood	29
I.V. catheters	10
Porcine graft	4
Foley tip	3
Homograft	1
Total	804

Table 24. Pseudomonas aeruginosa Total Isolates Sensitivity

Antibiotic	Resistant %	Resistant No.	Intermediate %	Intermediate No.	Sensitive %	Sensitive No.	Total Tested
Amikacin	39.55	318	23.63	190	36.82	296	804
Gentamicin	45.82	367	23.35	187	30.84	247	801
Tobramycin	79.27	631	1.01	8	19.72	157	796
Ticarcillin	32.77	254	10.84	84	56.39	437	775
Mezlocillin	48.61	366	5.18	39	46.22	348	753
Piperacillin	24.53	196	11.14	89	64.33	514	799
Moxalactam	43.93	351	46.06	368	10.01	80	799
Cefotaxime	51.56	412	44.81	358	3.63	29	799
Cefoperazone	34.56	273	16.58	131	48.86	386	790
Cefsulodin	17.92	143	2.63	21	79.45	634	798
Colistin	0.37	3	0.00	0	99.63	801	804
Sulfadiazine	84.68	669	3.04	24	12.28	97	790

Table 25. Comparison of Resistance Frequency between Pseudomonas aeruginosa Isolated in 1981 and 1982

Antibiotic	1981 Resistant/ Sensitive	1981 % Resistant	1982 Resistant/ Sensitive	1982 % Resistant	$X_2(1)$	P
Amikacin	299/266	52.9	318/486	39.6	23.95	<0.001
Gentamicin	428/135	76.0	367/434	45.8	124.00	<0.001
Tobramycin	362/40	90.1	631/165	79.3	21.80	<0.001
Ticarcillin	24/540	4.3	254/521	32.8	161.30	<0.001
Piperacillin	13/540	2.4	196/603	24.5	123.00	<0.001
Moxalactam	18/540	3.2	351/448	43.9	274.00	<0.001
Cefotaxime	82/97	45.8	412/378	51.6	2.34	<0.13*
Cefsulodin	8/556	8.0	143/655	17.9	91.30	<0.001
Colistin	4/561	0.7	3/801	0.4	0.73	<0.40*
Sulfadiazine	538/28	95.0	669/121	84.7	36.00	<0.001

* N.S.

Table 26. Sensitivity of Blood Isolates of Pseudomonas aeruginosa

Antibiotic	Resistant %	Resistant No.	Intermediate %	Intermediate No.	Sensitive %	Sensitive No.	Total Tested
Amikacin	44.8	13	20.7	6	34.5	10	29
Gentamicin	44.8	13	24.1	7	31.0	9	29
Tobramycin	86.2	25	0.0	0	13.8	4	29
Ticarcillin	31.0	9	3.5	1	65.5	19	29
Mezlocillin	39.1	9	0.0	0	60.9	14	23
Piperacillin	36.0	9	4.0	1	60.0	15	25
Moxalactam	28.0	7	60.0	15	12.0	3	25
Cefotaxime	36.0	9	64.0	16	0.0	0	25
Cefoperazone	40.0	10	0.0	0	60.0	15	25
Cefsulodin	24.0	6	4.0	1	72.0	18	25
Colistin	0.0	0	0.0	0	0.0	29	29
Sulfadiazine	100.0	25	0.0	0	0.0	0	25

Table 27. Sources of Group D Enterococcus Tested for Antibiotic Sensitivity

Source	Number of Isolates
Sputum	32
Blood	29
Biopsy	3
I.V. catheters	2
Swabs	2

Table 28. Group D Enterococcus Total Isolates Sensitivity

Antibiotic	Resistant %	Resistant No.	Intermediate %	Intermediate No.	Sensitive %	Sensitive No.	Total Tested
Amikacin	88.71	55	6.45	4	4.84	3	62
Gentamicin	31.82	21	51.52	34	16.67	11	66
Tobramycin	63.64	42	21.21	14	15.15	10	66
Ticarcillin	1.64	1	3.28	2	95.08	58	61
Mezlocillin	1.64	1	0.00	0	98.36	60	61
Piperacillin	1.61	1	1.61	1	96.77	60	62
Moxalactam	95.16	59	3.23	2	1.61	1	62
Cefotaxime	66.13	41	29.03	18	4.84	3	62
Cefoperazone	100.00	1	0.00	0	0.00	0	1
Cefsulodin	100.00	1	0.00	0	0.00	0	1
Colistin	0.00	0	0.00	0	100.00	1	1
Sulfadiazine	97.77	60	0.00	0	3.23	2	62
Methicillin	89.23	58	1.54	1	9.23	6	65
Cephalothin	62.30	38	32.79	20	4.92	3	61
Vancomycin	1.54	1	0.00	0	98.46	64	65

Table 29. Sources of Staphylococcus epidermidis Tested for Antibiotic Sensitivity

Source	Number of Isolates
Blood	14
Sputum	4
Contact plate	2
I.V. catheters	2
Swabs	2
Homograft	2
Urine	1

Table 30. Staphylococcus epidermidis Total Isolates Sensitivity

Antibiotic	Resistant %	Resistant No.	Intermediate %	Intermediate No.	Sensitive %	Sensitive No.	Total Tested
Amikacin	5.88	1	5.88	1	88.24	15	17
Gentamicin	15.38	4	11.54	3	73.08	19	26
Tobramycin	30.77	8	3.85	1	65.38	17	26
Ticarcillin	6.25	1	0.00	0	93.75	15	16
Mezlocillin	6.25	1	0.00	0	93.75	15	16
Piperacillin	5.88	1	5.88	1	88.24	15	17
Moxalactam	47.06	8	47.06	8	5.77	1	17
Cefotaxime	17.65	3	23.53	4	58.82	10	17
Sulfadiazine	64.71	11	5.88	1	29.41	5	17
Methicillin	23.08	6	3.85	1	73.08	19	26
Cephalothin	0.00	0	5.88	1	94.12	16	17
Vancomycin	0.00	0	0.00	0	100.00	26	26

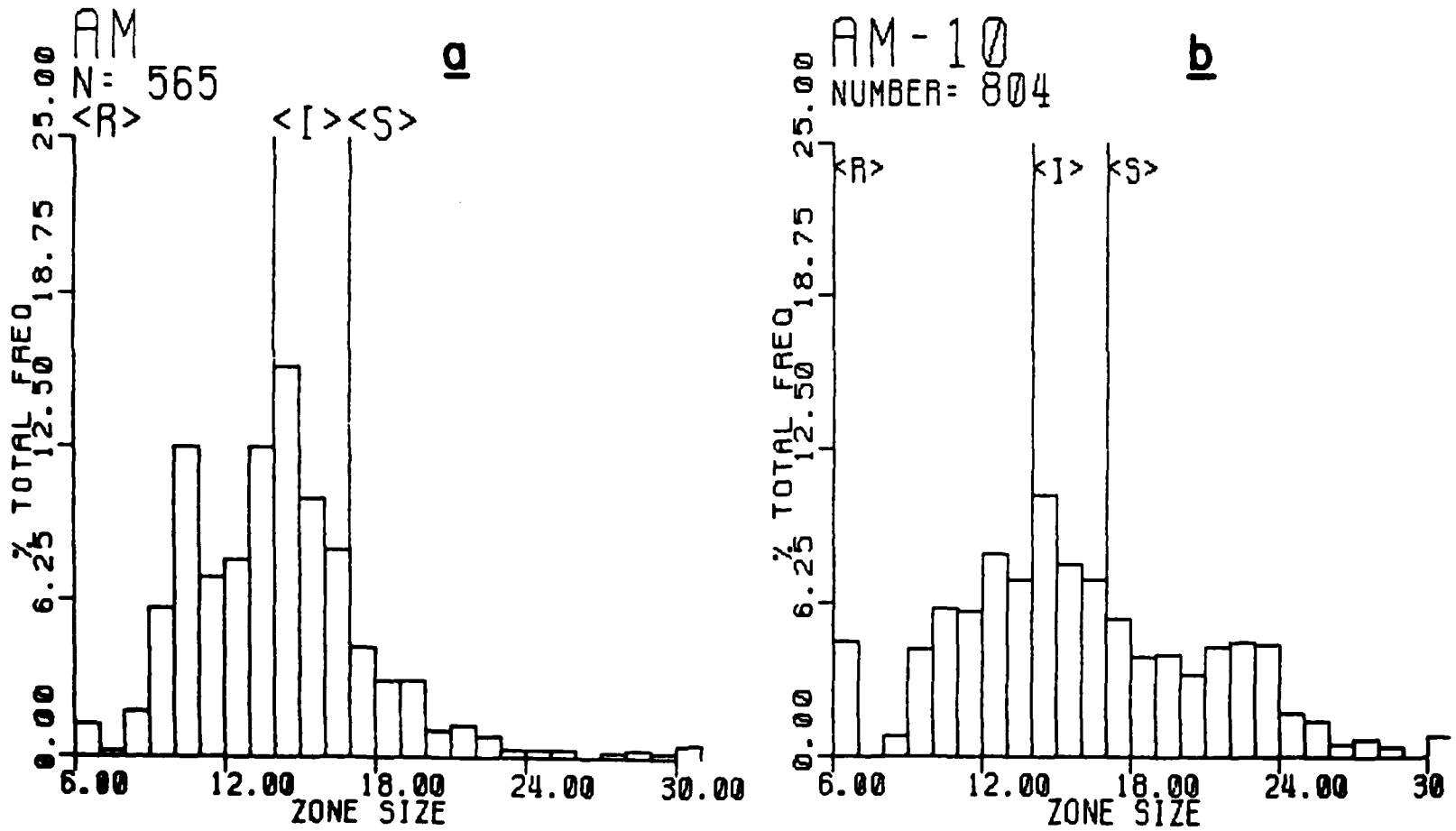

Figure 13. Histogram displays of the distribution of zones of inhibition of Pseudomonas aeruginosa using amikacin (10 µg) disc. Data from FY 81 = a, data from FY 82 = b.

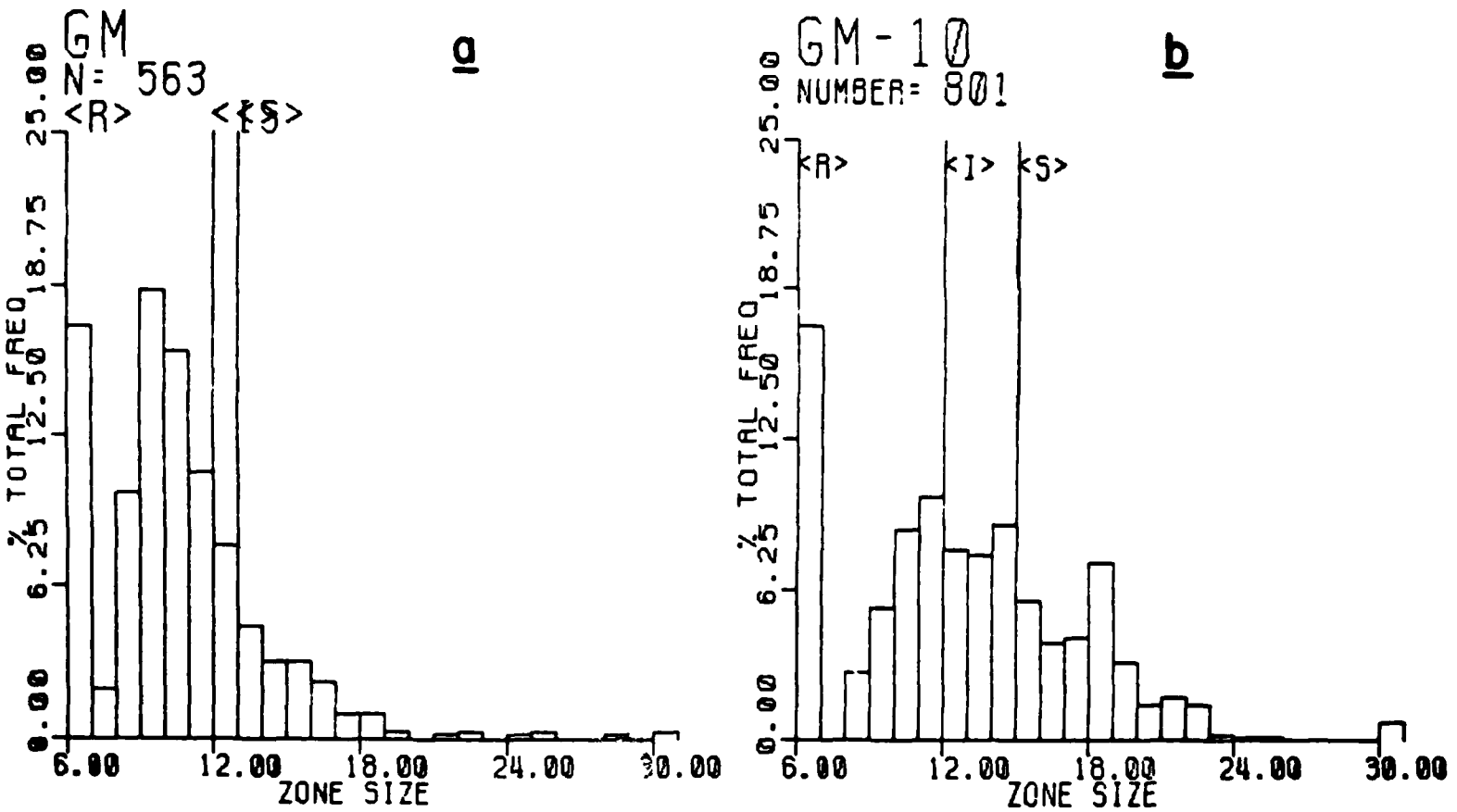

Figure 14. Histogram displays of the distribution of zones of inhibition of Pseudomonas aeruginosa using gentamicin (10 µg) disc. Data from FY 81 = a, data from FY 82 = b.

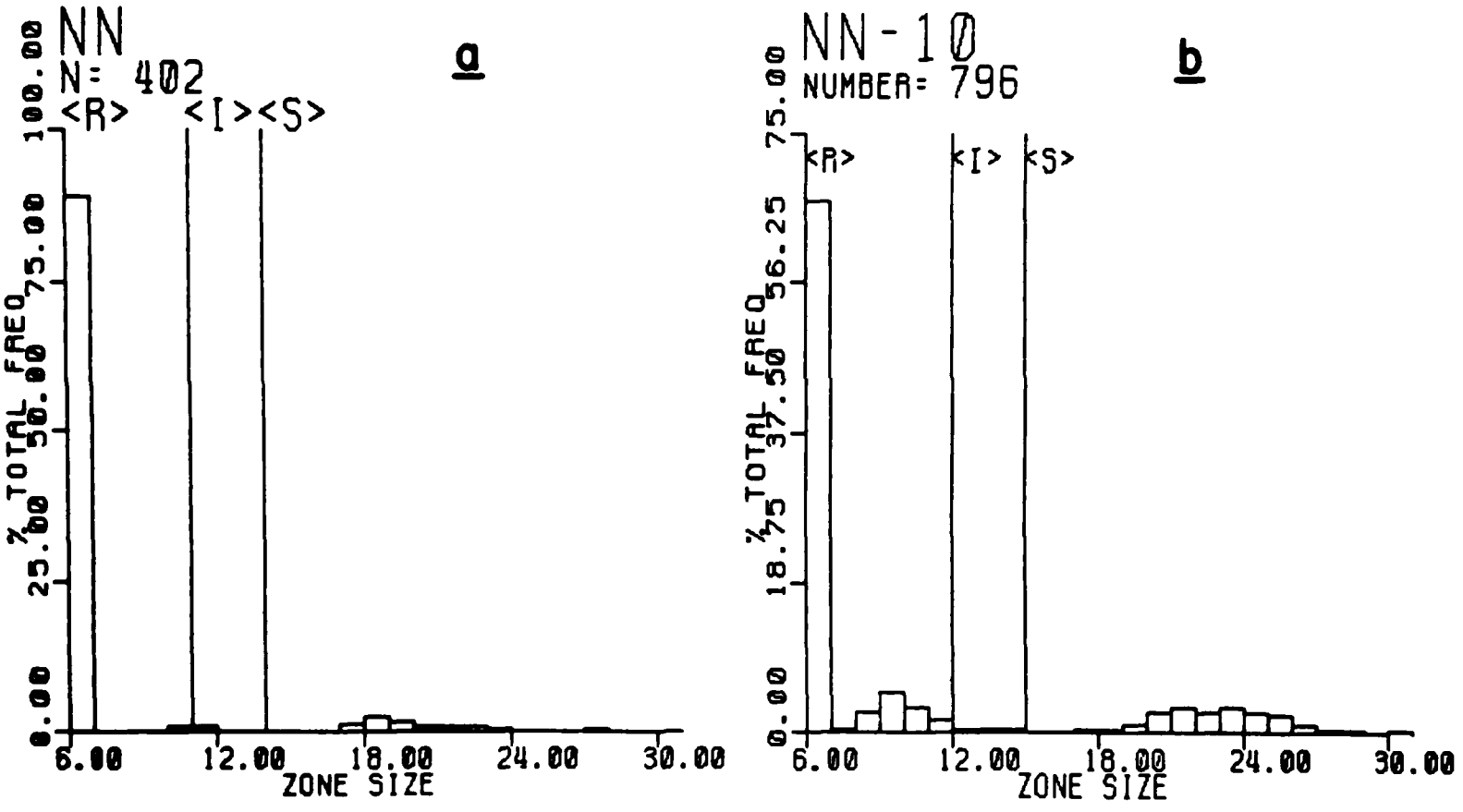

Figure 15. Histogram displays of the distribution of zones of inhibition of Pseudomonas aeruginosa using tobramycin (10 µg) disc. Data from FY 81 = a, data from FY 82 = b.

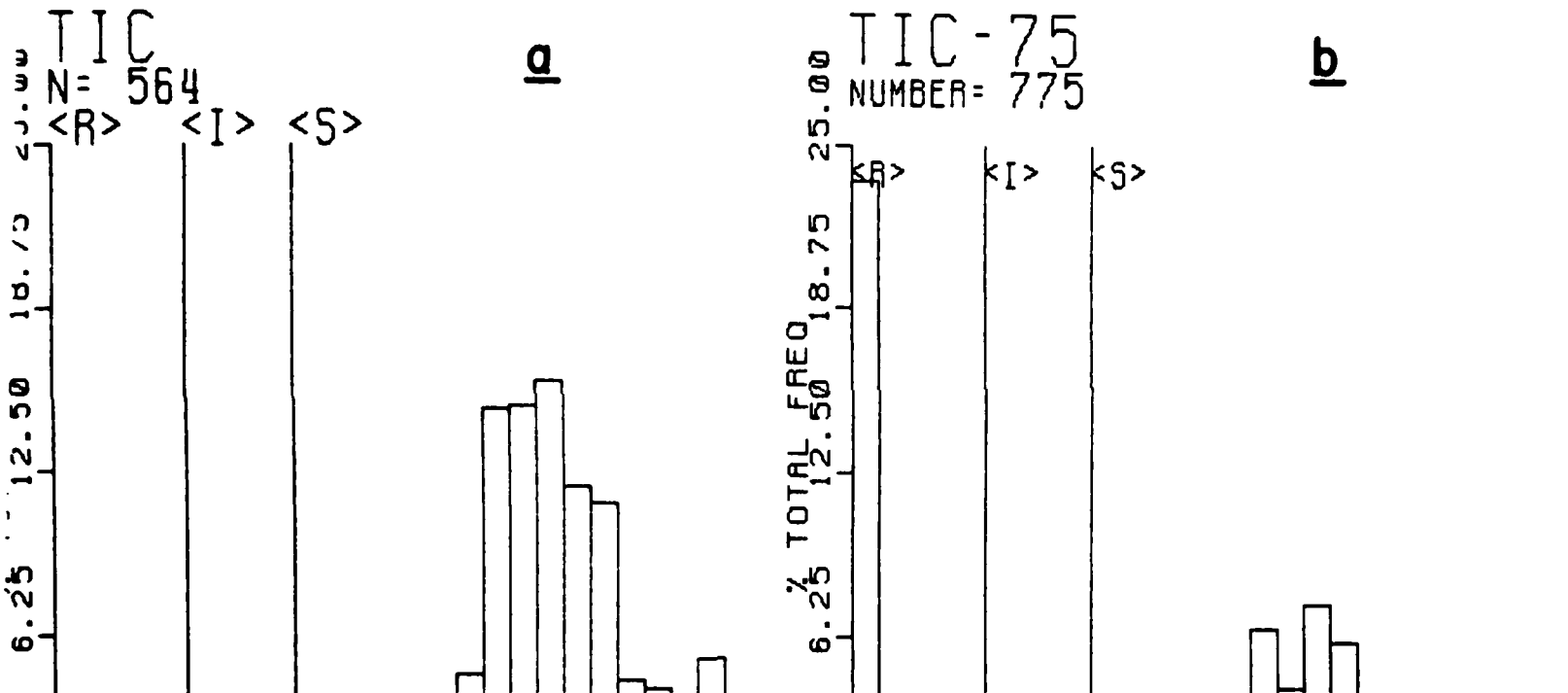

Figure 16. Histogram displays of the distribution of zones of inhibition of Pseudomonas aeruginosa using ticarcillin (75 µg) disc. Data from FY 81 = a, data from FY 82 = b.

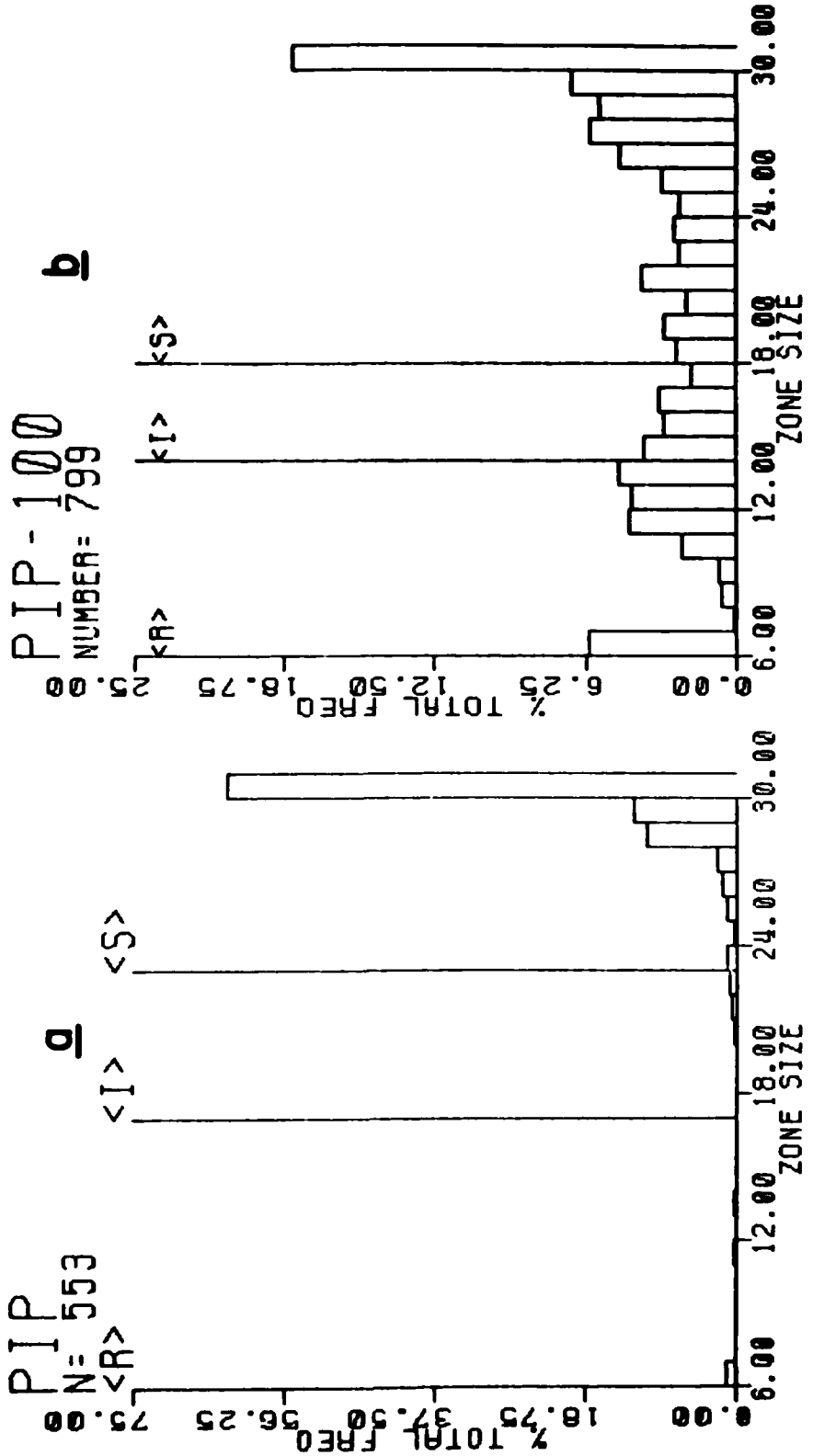

Figure 17. Histogram displays of the distribution of zones of inhibition of Pseudomonas aeruginosa using piperacillin (100 µg) disc. Data from FY 81 = a, data from FY 82 = b.

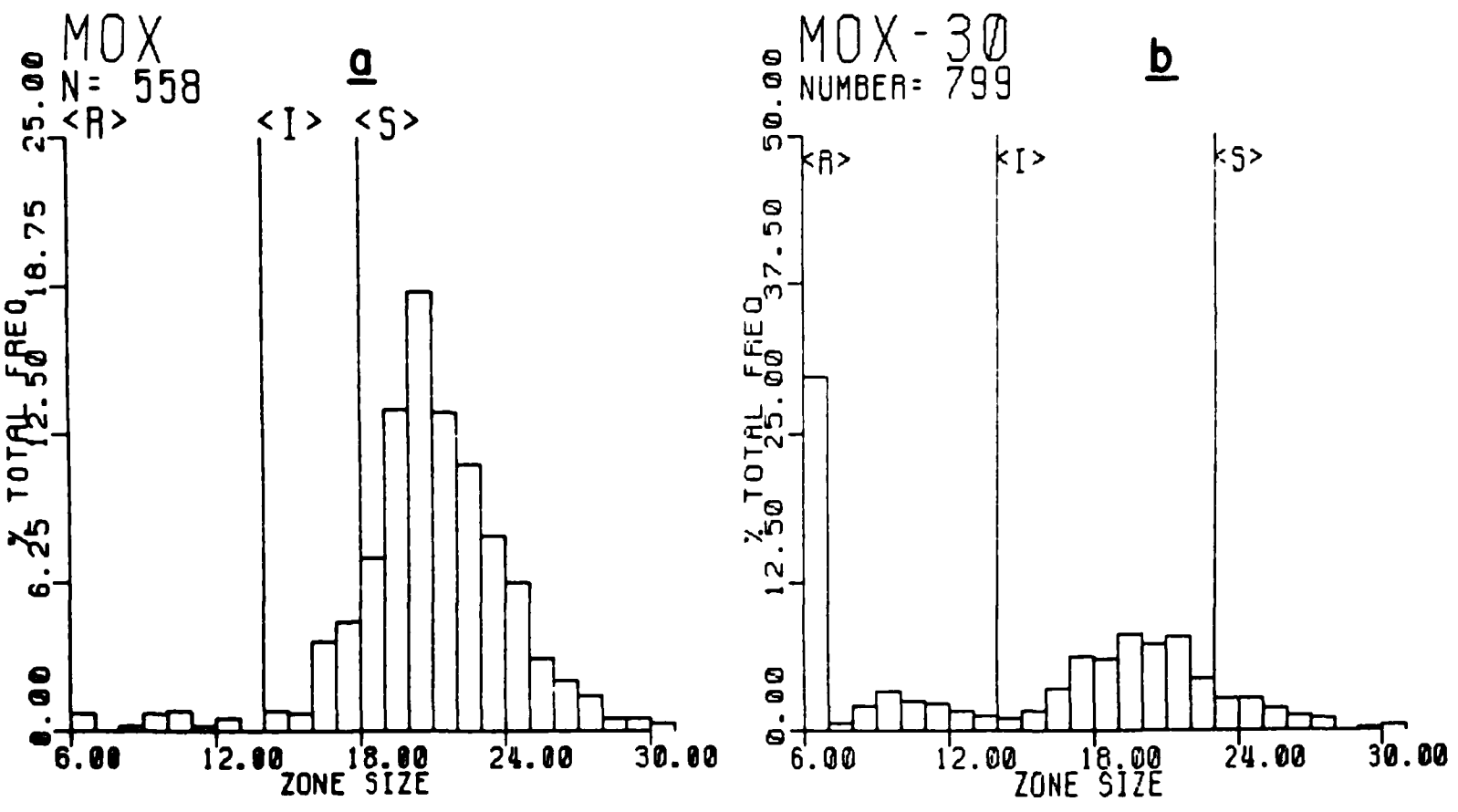

Figure 18. Histogram displays of the distribution of zones of inhibition of Pseudomonas aeruginosa using moxalactam (30 µg) disc. Data from FY 81 = a, data from FY 82 = b.

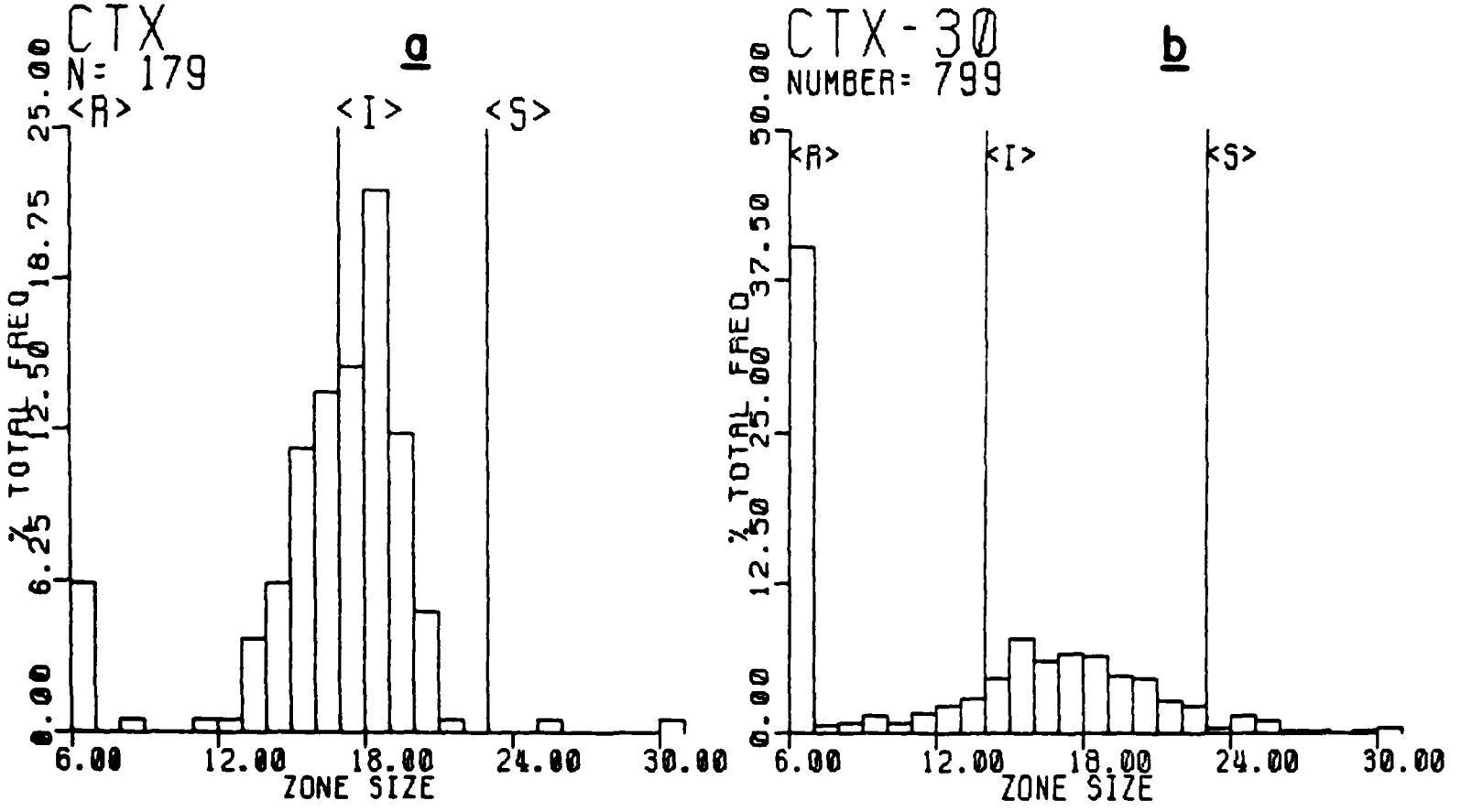

Figure 19. Histogram displays of the distribution of zones of inhibition of Pseudomonas aeruginosa using cefotaxime (30 µg) disc. Data from FY 81 = a, data from FY 82 = b.

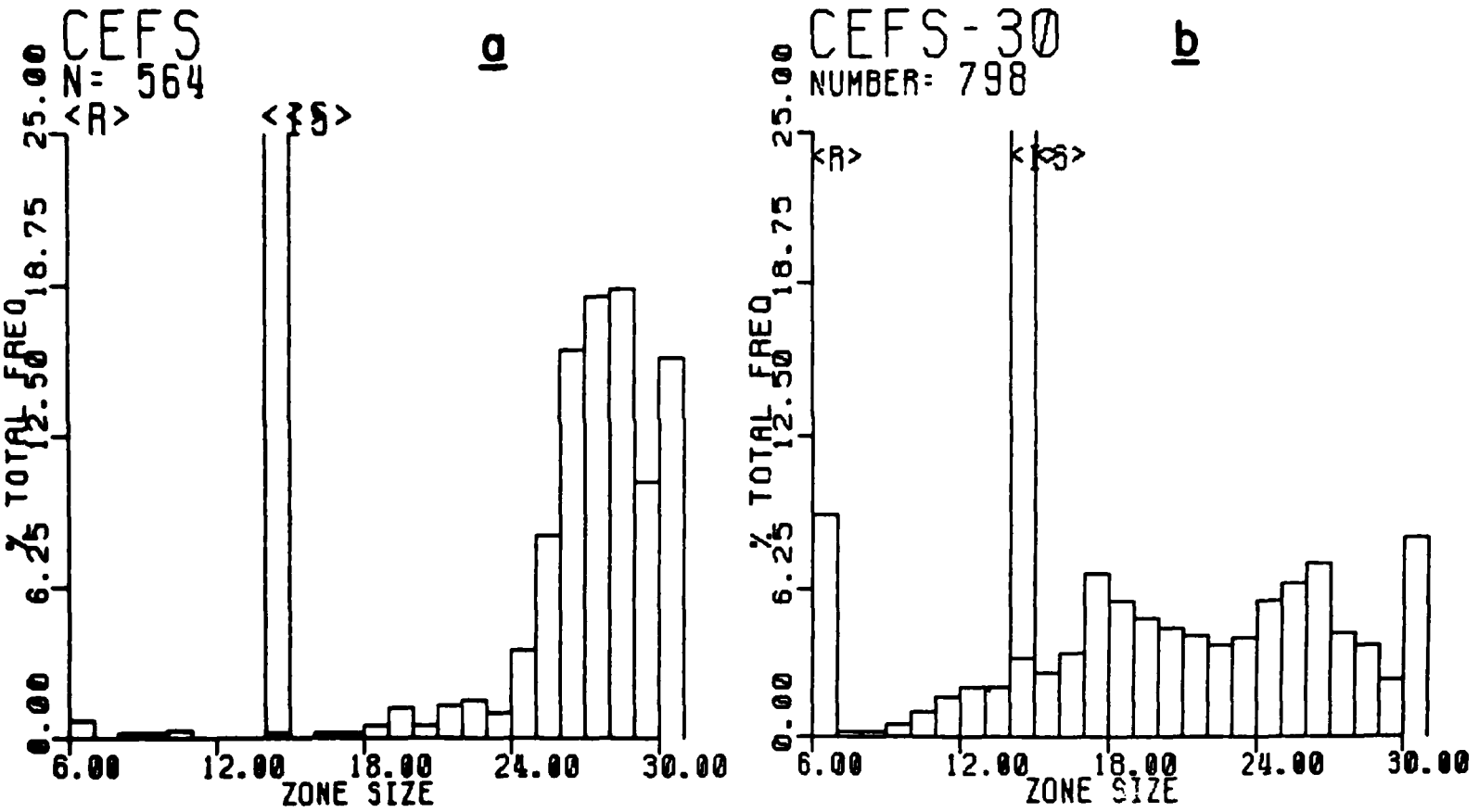

Figure 20. Histogram displays of the distribution of zones of inhibition of Pseudomonas aeruginosa using cefsulodin (30 µg) disc. Data from FY 81 = a, data from FY 82 = b.

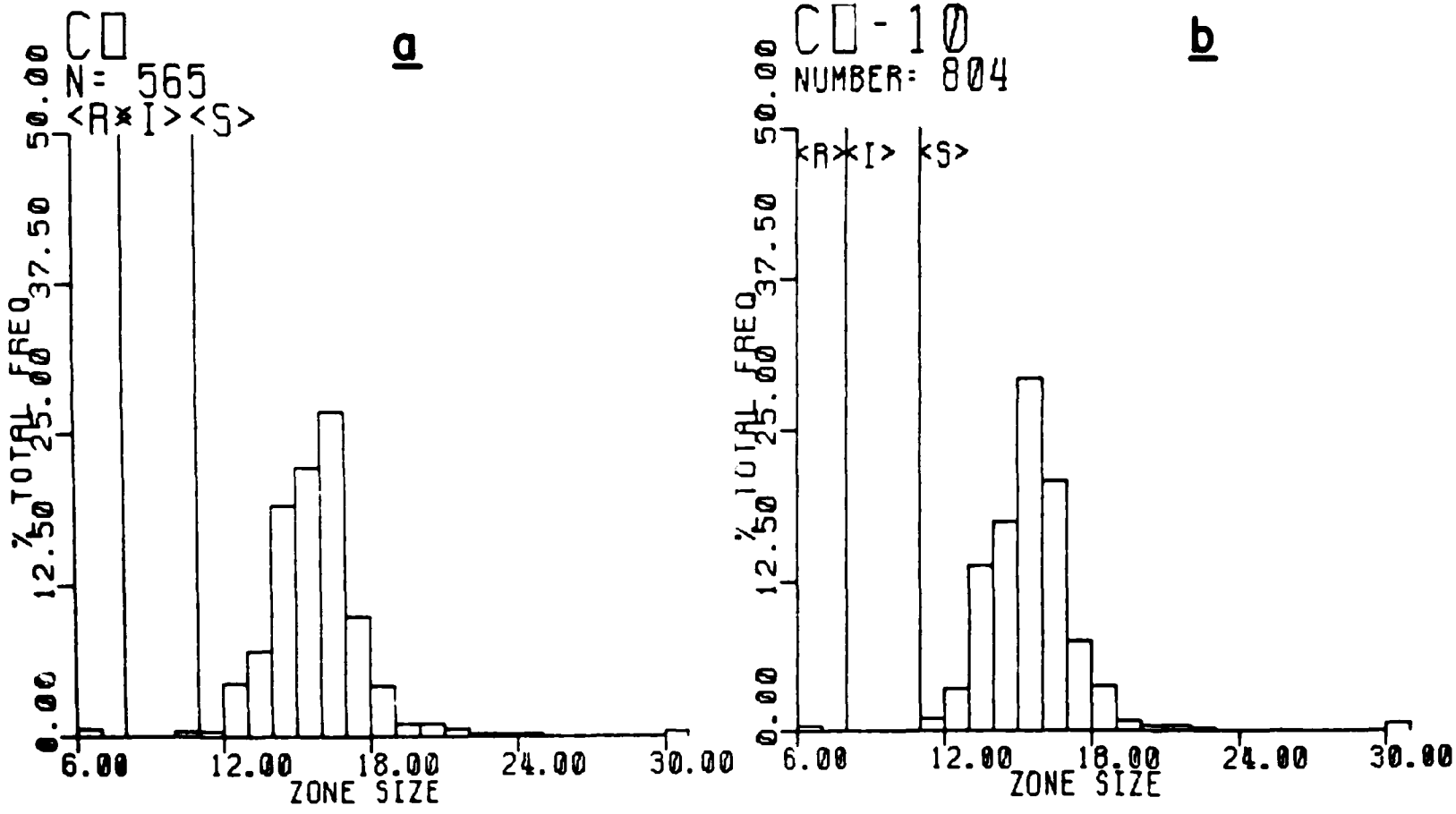

Figure 21. Histogram displays of the distribution of zones of inhibition of Pseudomonas aeruginosa using colistin (10 µg) disc. Data from FY 81 = a, data from FY 82 = b.

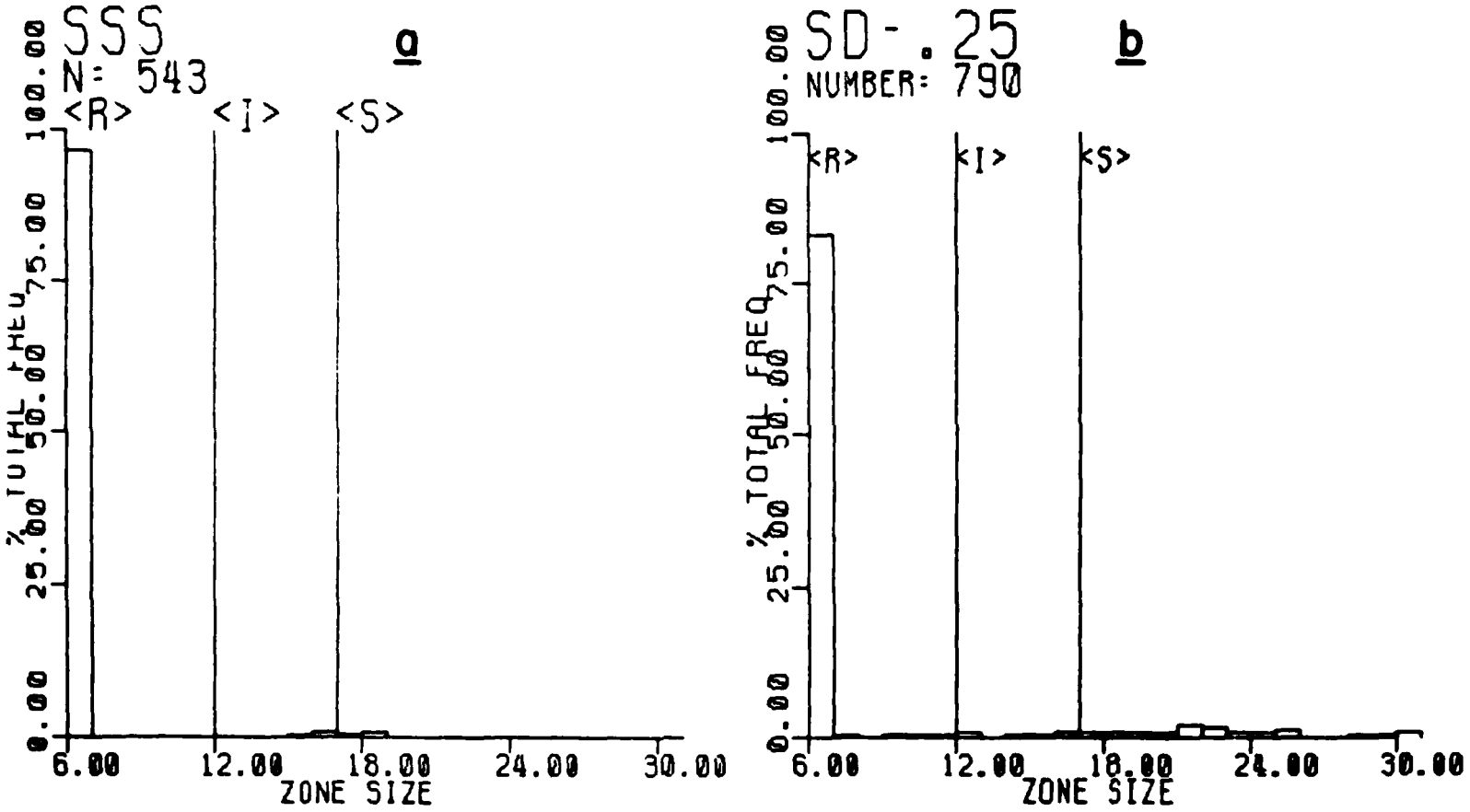

Figure 22. Histogram displays of the distribution of zones of inhibition of Pseudomonas aeruginosa using triple sulfonamide (250 µg) SSS disc or sulfadiazine (250 µg) disc. Data from FY 81 = a, data from FY 82 = b.

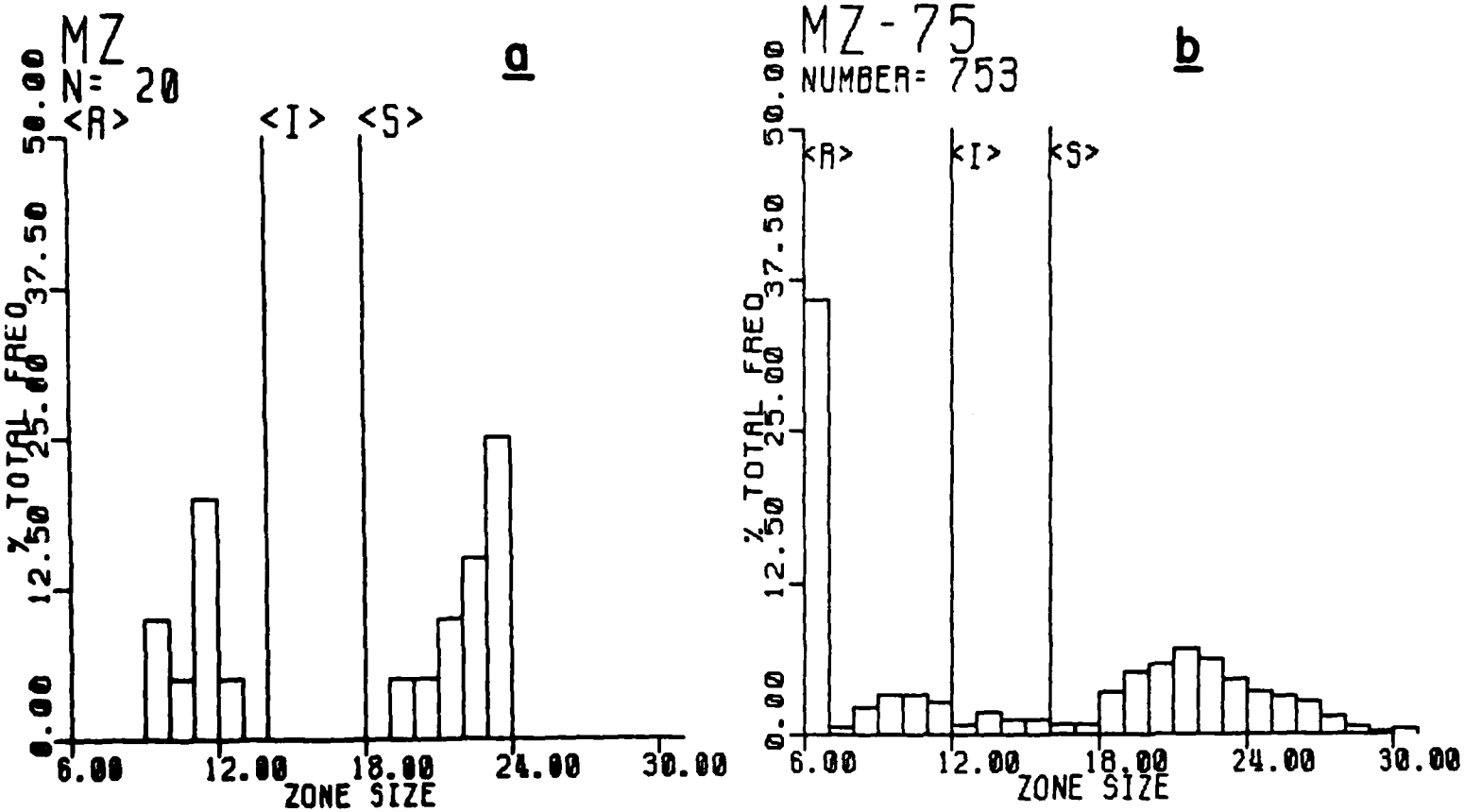

Figure 23. Histogram displays of the distribution of zones of inhibition of Pseudomonas aeruginosa using mezlocillin (75 µg) disc. Data from FY 81 = a, data from FY 82 = b.

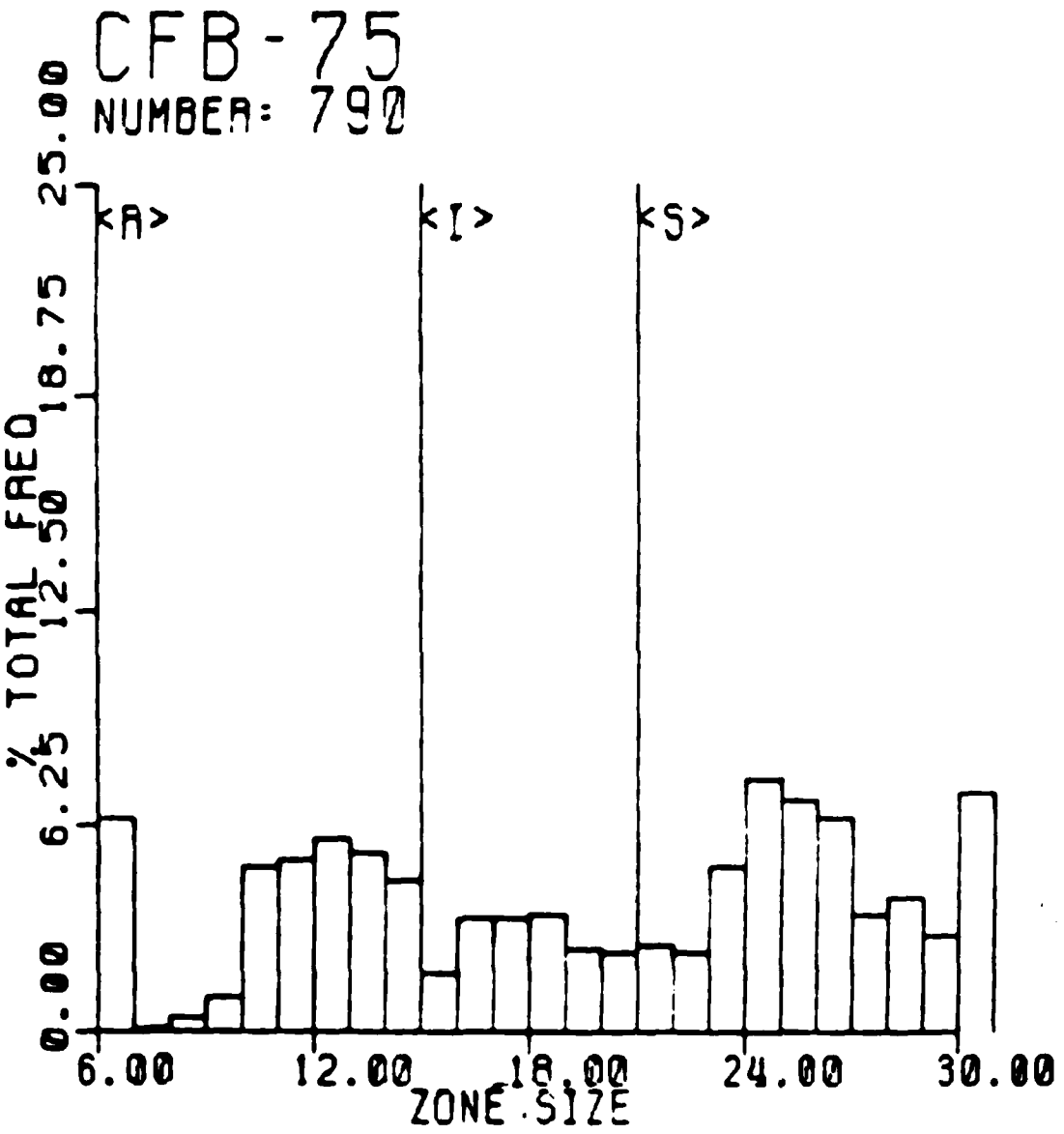

Figure 24. Histogram display of the distribution of zones of inhibition of Pseudomonas aeruginosa using cefoperazone (75 µg) disc.

KLEBSIELLA PNEUMONIAE SENSITIVITIES

A total of 146 isolates from 32 patients were examined. The source distributions are presented in Table 31. Antibiotic sensitivity data are presented in Table 32. All strains were sensitive to the tested aminoglycosides. The increased activity of the mezlocillin and piperacillin over ticarcillin is obvious in this sample. High frequency sulfonamide resistance was also observed. Histogram displays are not presented. The blood isolation frequency was too small to test against the total sample.

PUBLICATIONS

Sokel PA, Inglewski BH, Hager TA, Sadoff GC, Cross AS, McManus AT, Farber BF, Inglewski WJ: Production of Exoenzyme S by clinical isolates of Pseudomonas aeruginosa. Infect Immun 34:147-153, 1981.

PRESENTATIONS

None.

For annual readers who may not have other communication routes with this Institute, I unfortunately must report the death of Dr. Robert B. Lindberg, 3 November 1982. His loss will be keenly felt by all his friends and everyone active in the field of surgical microbiology.

Table 31. Sources of Klebsiella pneumoniae Tested for Antibiotic Sensitivity

Source	Number of Isolates
Sputum	106
Blood	14
Urine	14
I.V. catheters	5
Biopsy	2
Foley tip	2
Swabs	2
Contact plate	1

Table 32. Klebsiella pneumoniae Total Isolates Sensitivity

Antibiotic	Resistant %	Resistant No.	Intermediate %	Intermediate No.	Sensitive %	Sensitive No.	Total Tested
Amikacin	0.00	0	0.00	0	100.00	146	146
Gentamicin	0.00	0	0.00	0	100.00	146	146
Tobramycin	0.00	0	0.00	0	100.00	146	146
Ticarcillin	51.41	73	40.85	58	7.75	11	142
Mezlocillin	9.70	13	8.21	11	82.09	110	134
Piperacillin	13.19	19	4.86	7	81.94	118	144
Moxalactam	1.39	2	2.78	4	95.83	138	144
Cefotaxime	0.69	1	0.69	1	98.61	142	144
Cefoperazone	2.08	3	6.94	10	90.97	131	144
Cefsulodine	89.61	69	1.30	1	9.09	7	77
Colistin	1.37	2	0.00	0	98.63	144	146
Sulfadiazine	65.97	95	0.69	1	33.33	48	144

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA 0G 6969	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BB	302
b. CONTRIBUTING				
c. XXXXXXXX	STOG - 80	- 7.2:5		

11. TITLE (Procede with Security Classification Code)
(U) The Study of Metabolism and Nutritional Effects of Burn Injury in Soldiers

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 10	Cont	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	5.5	370
	CURRENT 83	5.5	330

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Institute of Surgical Research
ADDRESS: Fort Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL
NAME: Basil A. Pruitt, Jr, MD, COL, MC
TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research
Surgical Study Branch
ADDRESS: Fort Sam Houston, Texas 78234

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Cleon W. Goodwin, Jr., MD
TELEPHONE: 512- 221-5712
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Nitrogen Balance; (U) Burn Injury; (U) Computer Surveillance; (U) Metabolism; (U) Humans; (U) Animal Model

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To identify afferent and efferent mediators of postinjury hypermetabolism and altered thermoregulation in burned soldiers. To describe the effects of thermal injury on endocrine function and metabolism of proteins, carbohydrates, and fats. To establish optimal nutritional support for thermally injured patients by computer analysis of daily balance studies.

24. (U) Environmental chambers serve as an experimental laboratory for monitoring thermoregulatory and metabolic alterations of burned patients and burned animals. An injured animal model has been developed to characterize the time course of postinjury hypermetabolism and the associated changes in substrate delivery following trauma. Isolation of adipocytes from fat biopsies and arterial and venous blood analyses are conducted in both patients and animal models to measure the fluxes of various substrates from fatty depots and across different regional beds. Pertinent clinical data from daily clinical assessment and laboratory studies are stored in computerized data files for continuous on-line analysis of nutritional therapy.

25. (U) 8110 - 8209. The isolated adipocyte has been chosen as a controlled environment for determining the function of adipose tissue in normal and injured patients. The preliminary studies have demonstrated the proper sampling approach in patients and have resulted in

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE DD FORMS 1498A 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

improvement of the technique for obtaining such specimens. With the aid of the recently developed continuous computer graphics program, and with the verification of this program by direct measurement of metabolic rate in an environmental chamber, the efficacy of several commonly used methods for estimating nutritional requirements has been completed. These abbreviated methods all result in serious underestimation of nutritional requirements in critically ill patients. Partly because of the presence of a rumen, the thermally injured goat has been found to be an unreliable model of postburn hypermetabolism. Current studies utilize a monogastric animal model, the pig.

ANNUAL PROGRESS REPORT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

REPORT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF BURN INJURY IN SOLDIERS -- METABOLIC AND THERMOREGULATORY ADJUSTMENTS TO BURN INJURY: A PIG MODEL

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1981 - 30 September 1982

Investigators:

Louis H. Aulick, Ph.D., LTC, MSC
Edwin W. Hander, M.S.
Hartmut Arnhold, A.E.
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

REPORT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF BURN INJURY IN SOLDIERS -- METABOLIC AND THERMOREGULATORY ADJUSTMENTS TO BURN INJURY: A PIG MODEL

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1981 - 30 September 1982

Investigators: Louis H. Aulick, Ph.D, LTC, MSC
Edwin W. Hander, M.S.
Hartmut Arnhold, A.E.
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

This report covers initial work in the development of a large animal model (miniature swine) of postburn hypermetabolism. It describes the conditioning phase of the study and includes data from control and experimental studies in three of the scheduled five animals. The resting metabolic rates of control animals were 63.5 ± 1.2 and 62.2 ± 1.3 Watts/m^2 (mean $\pm$ SE) at ambient temperatures of 25 and 30°C. When the temperature of the chamber was reduced below this thermoneutral zone, energy turnover rose in a predictable manner. The lower critical temperature was near 25°C for the three pigs. Metabolic heat production of two pigs was elevated 30-40 percent above normal resting levels on the first day after they received thermal burns covering 21 and 27 percent total body surface burns. This level of hypermetabolism was sustained throughout the first week. Associated with the increased heat production was a transient rise in core temperature, but by the end of one week, both injured animals were normothermic. Additional studies are currently underway which will characterize the metabolic and thermoregulatory changes in this model over the next two weeks. When completed, the project is designed to determine if the increase in heat production develops because the injured animal is cold.

Temperature
Thermoregulatory response
Metabolic response

METABOLIC AND THERMOREGULATORY ADJUSTMENTS TO BURN INJURY: A PIG MODEL

INTRODUCTION

Thermal injury causes well recognized increases in metabolic heat production and body temperatures. Most of the evidence to date suggests that the hypermetabolic response is a reflection of the added energy requirements of the wound (1), but a persistent school of thought argues that the extra heat production is in response to the accelerated rate of evaporative heat loss across the surface wound (2). This controversy has two possible origins. First, the basic interaction between metabolism and body temperature cannot be fully explored in burn patients due to the constraints of clinical research. Second, an appropriate animal model has not been developed. Small, fur-bearing animal burn models used in the past are not entirely appropriate, since their size and external insulation make them considerably different (in a thermoregulatory sense) from man.
regulatory sense) from man.

The pig may be the best, non-primate burn model for this particular study, since it has both proven to be a good model of several human physiological systems (3,4) and has demonstrated metabolic and endocrine responses to burn injury similar to those observed in patients (5). From a thermoregulatory standpoint, the large pig has a number of positive features. Like man, it is essentially hairless and must rely on internal forms of insulation to protect against heat loss. In addition, the larger animal has a surface-to-mass ratio nearer to that of man. Finally, pigs have no brown fat. Consequently, the metabolic response to cold stress should involve thermogenic mechanisms more like the patient's than many of the small animal models previously studied.

This study is designed to determine the effects of thermal injury on the relationship between metabolic heat production and body temperature of miniature swine. It will determine if resting metabolism is increased following burn injury and, if so, whether the extra heat production can be eliminated by increasing ambient temperatures. In humans, the metabolic response to burn injury is temperature-sensitive but not

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1. Aulick LH, Hander EH, Wilmore DW, and Mason AD Jr: The relative significance of thermal and metabolic demands on burn hypermetabolism. J Trauma 19:559, 1979.

2. Danielsson U, Arturson G, and Wennberg L: The elimination of hypermetabolism in burn patients. Burns 2:110, 1976.

3. Bustad L: Pigs in the laboratory. Sci Amer 214:94, 1966.

4. McClellan RO: Application of swine in biomedical research. Lab Anim Care 18:120, 1968.

5. Wachtel TL, Shuck JM, Eaton RP, Schade D, and Shuck LW: Glucagon, insulin and glucose relationships in a porcine experimental burn model. J Surg Res 24:70, 1978.

temperature dependent. This has not been the case in some small animals where the hypermetabolism is abolished by environmental heating (6-8).

The second issue to be addressed is whether the increase in heat production is associated with an increase in body heat content. If so, this study will determine if the injured animal appears to be thermoregulating in a normal manner around an elevated central reference temperature (a febrile response similar to the patient) or whether the hyperthermia simply reflects an imbalance between heat production and heat loss.

METHODS

Animals. The animals selected for study are one-year-old, female, miniature swine (Pitman-Moore strain) weighing 40-60 kg. A total of five animals will be studied. Upon arrival at the laboratory, they are housed in outdoor runs, fed commercial pig feed (20 grams/kg body weight/day) and given water ad libitum. Three weeks prior to study, the animals are moved indoors where ambient temperature can be maintained between 24° and 27°C. Here, they are quartered in individual pens for the remainder of the experiment.

Study Design. The first phase of each study is devoted to animal conditioning. This includes the development of a general acceptance of both the laboratory and a large respiration chamber. Chamber conditioning sessions are performed between 2000 and 0700 hours, five nights per week, until metabolic measurements indicate that all animals have reached their lowest level of activity. All conditioning runs are conducted at a chamber temperature of 25°C and relative humidity between 40-50 percent. Rectal and six skin temperatures are taken immediately after each run. The animals are then fed their full daily ration and left essentially undisturbed for the rest of the day.

Once the control values for metabolic heat production and body temperature have been established in this thermoneutral environment, chamber temperature is lowered to 20°C, and two to three overnight studies are performed on each animal. This process is repeated at chamber temperatures of 15°, 10°, 5° and 30°C in order to define the normal animal's thermoneutral zone and lower critical temperature.

Following these control studies, the pigs are anesthetized with a mixture of methoxyfluorane and 100 percent oxygen. Control femoral venous blood samples (30 ml) are drawn and a small temperature radiotransmitter implanted in the midline of the abdomen between

6. Caldwell FT Jr, Osterholm JL, Sower ND, and Moyer CA: Metabolic response to thermal trauma of normal and thyroprivic rats at three environmental temperatures. Ann Surg 150:976, 1959.
7. Farkas LG, McCain WG, Birch JR, and James J: The effects of four different chamber climates on the oxygen consumption and healing of severely burned rats. J Trauma 13:911, 1973.
8. Herndon DN, Wilmore DW, and Mason AD Jr: Development and analysis of a small animal model simulating the human postburn response. J Surg Res 25:394, 1978.

the linea alba and peritoneum. Additional control studies begin one week post surgery and continue until a well defined metabolic-core temperature relationship has been established. The animals are then anesthetized as before and their backs and both sides shaved. While in a surgical plane of anesthesia, a third degree flame burn is created over the shaved area covering 20-30 percent of the total body surface. These animals are allowed to recover spontaneously without fluid or electrolyte administration. No topical or systemic antibiotic therapy is given.

The injured animals are studied on alternate days for the next three weeks. Chamber temperature remains at 25°C for the first 7-10 days post injury or until the anticipated hypermetabolic response is well established. Ambient temperature is then varied as before to identify any change in the thermoneutral zone and lower critical temperature of the burned animal. The degree of hot or cold stress is limited to environments in which the animal can maintain a stable core temperature. Core and surface temperatures are determined daily.

At the end of the study, femoral venous blood samples are drawn and the animals sacrificed. Wound biopsies are obtained for histological examination.

Study Methods. Metabolic heat production is estimated from the pig's respiratory gas exchange while confined in an hermetically sealed chamber. The operation of this chamber has been explained elsewhere (9), but basically, it is a fully automated, open and closed system where a series of metabolic measurements can be performed, separated only by brief periods of chamber ventilation. The length of an individual run depends on the metabolism of the animal, since the run is terminated and the chamber ventilated when CO_2 concentration reaches 0.85 percent. All metabolic measurements are conducted during the evening hours when the animal is postabsorptive and quiet.

Core temperatures are monitored in two ways. First, rectal temperatures are taken with a standard glass thermometer immediately after each overnight run. Second, peritoneal temperature is monitored at frequent intervals during each overnight run through the use of the implanted radiotransmitter (Model LM, Mini-Mitter). Surface temperatures are also measured in two ways. Immediately after each experiment, six skin temperatures (face, shoulder, back 1, back 2, hip and abdomen) are measured with a hand-held thermistor probe and recorded on a Tektronix 501 digital voltmeter. An average of these six values represents the mean skin temperature for the animal. Rectal and these surface temperatures are collected in the laboratory at an ambient temperature of 24-27°C. In addition, the temperatures of the ear

9. Aulick LH, Hander EW, Arnhold H, and Mason AD Jr: A new approach to the study of the hypermetabolic response to thermal injury. Annual Progress Report, U.S. Army Institute of Surgical Research 1 October 1980 - 30 September 1981.

pinna and the skin over the middle of the back are monitored continuously while the pig is in the chamber. This is accomplished through the use of thermistors attached to the skin in these areas and recorded on a small analog-to-digital recorder (Solicorder) carried on the animal's back.

RESULTS

Control Studies. To date, three pigs have been conditioned to overnight chamber confinement and control studies have been conducted at ambient temperatures of 10, 15, 20, 25 and 30 degrees centigrade. The conditioning phase required about one month before metabolic rates reached an acceptable steady state level. A total of 69 control studies were then performed at the five chamber temperatures. Over the five-month period, body weight of the pigs increased from 40-45 kg to 70-75 kg.

In general, even the well-conditioned animals roamed around the chamber for the first 30-60 minutes before lying down to rest. They usually then remained quiet until about 0400-0500 hours the next morning. In order to avoid these active periods and provide an estimate of resting metabolic rate, only the data collected between the hours of 2200 and 0400 are reported.

Resting metabolic rate of the uninjured controls was the same in the 25^{0} and 30^{0}C environments (63.5 $\pm$ 1.2 and 62.2 $\pm$ 1.3 Watts/m^{2}; mean $\pm$ SE), but it rose in a predictable manner when the chamber temperature was reduced below 25^{0}C (Figure 1). In general, core temperature (recorded from the peritoneal surface) drifted down slowly during the night, but the rate and magnitude of central body cooling was not apparently determined by environmental temperature (Figure 2). At a chamber temperature of 25^{0}C, back skin temperature dropped slowly during the experiment while that of the ear pinna fluctuated markedly (Figure 3). In the 30^{0}C chamber, ear and back surface temperatures were elevated, less widely separated and more stable over night (Figure 4).

Burn Studies. Two pigs have received 21 (Pig 3) and 27 (Pig 1) percent total body surface burns and have been studied in the 25^{0}C chamber on alternate nights for one week. Resting metabolic heat production rose sharply after injury and remained 30-40 percent above control levels during the first week (Figure 5). Peritoneal temperature of both pigs was also elevated on the first postburn day but returned to normal by the end of the first week (Figure 6). The temperature of the burn wound on the back was elevated above that of uninjured back skin of the same animal before injury, but the ear temperature of the burned animals remained depressed (figures 3 and 7).

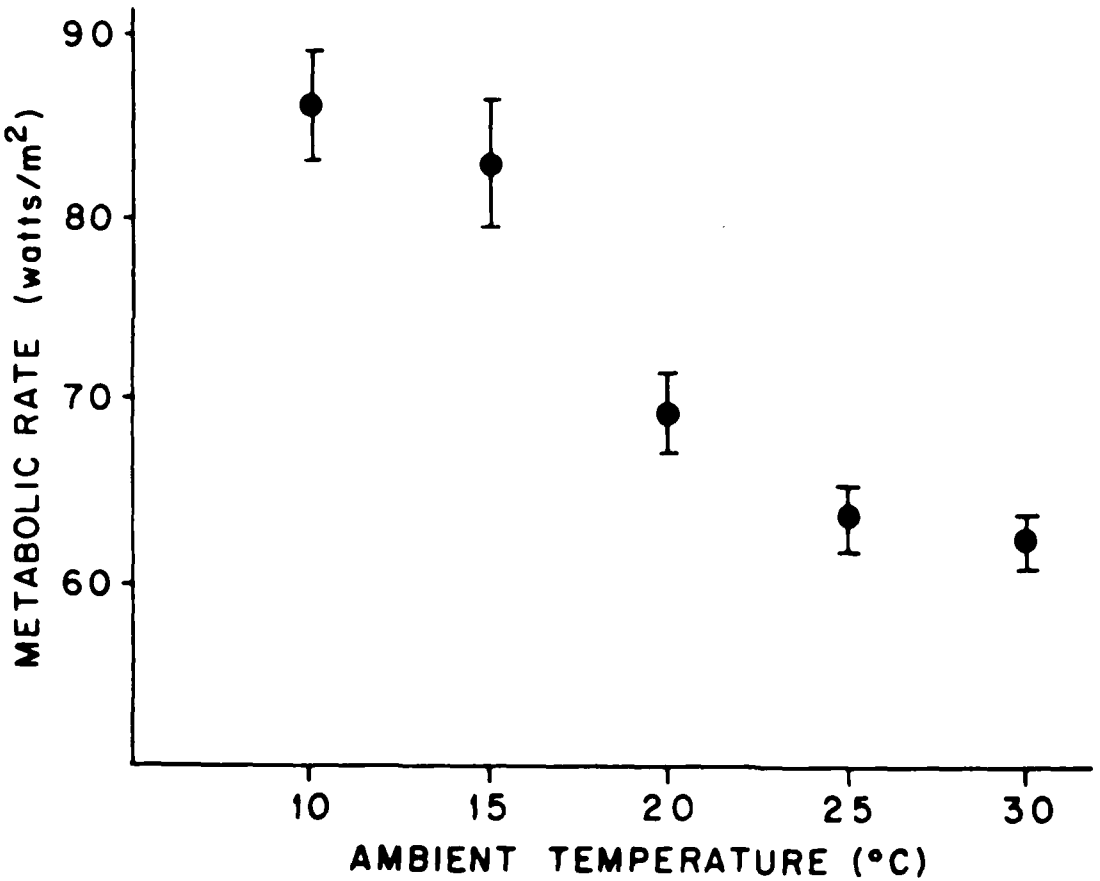

Figure 1. The effects of ambient environment on the metabolic heat production of three uninjured pigs.

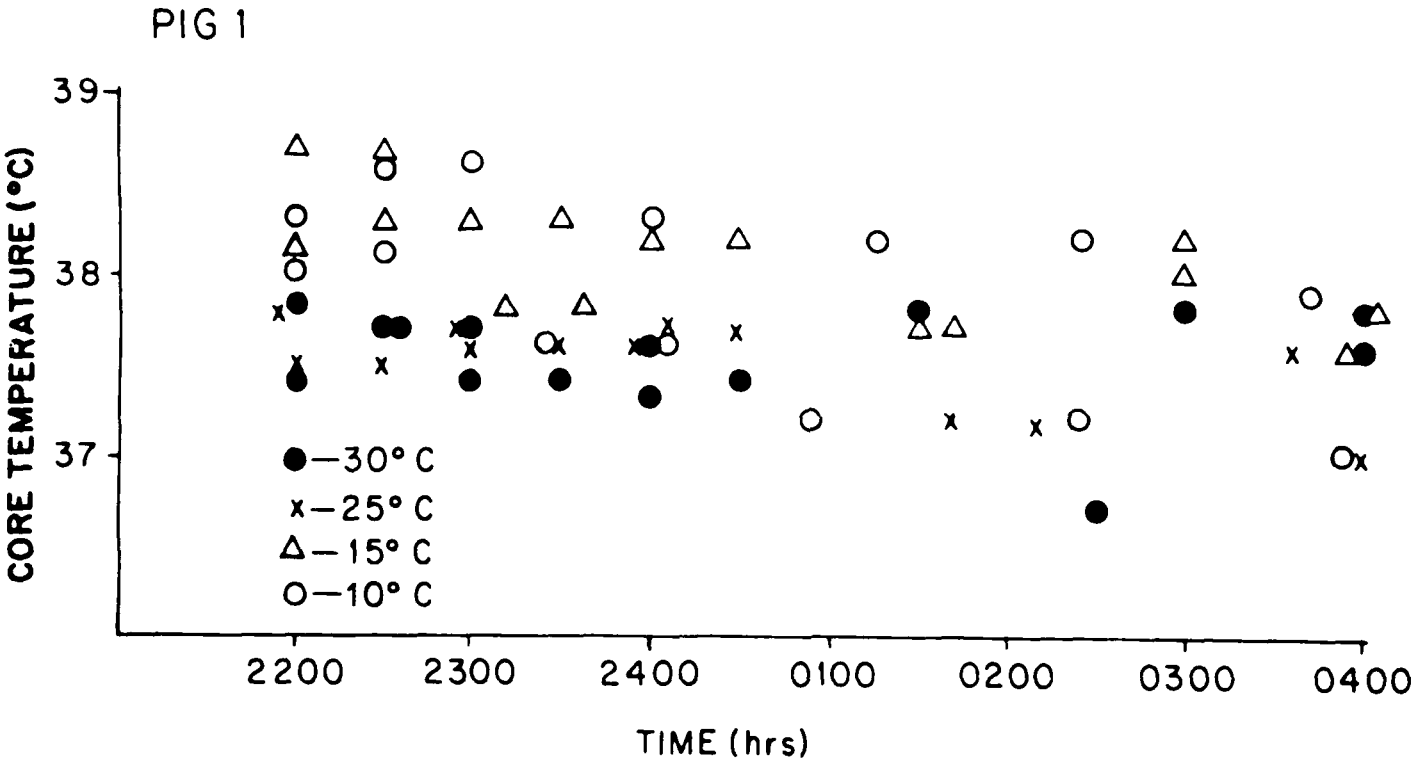

Figure 2. The gradual decline in peritoneal temperature of an uninjured pig during two typical overnight studies at different chamber temperatures (● at 30°C, X at 25°C, Δ at 15°C and O at 10°C).

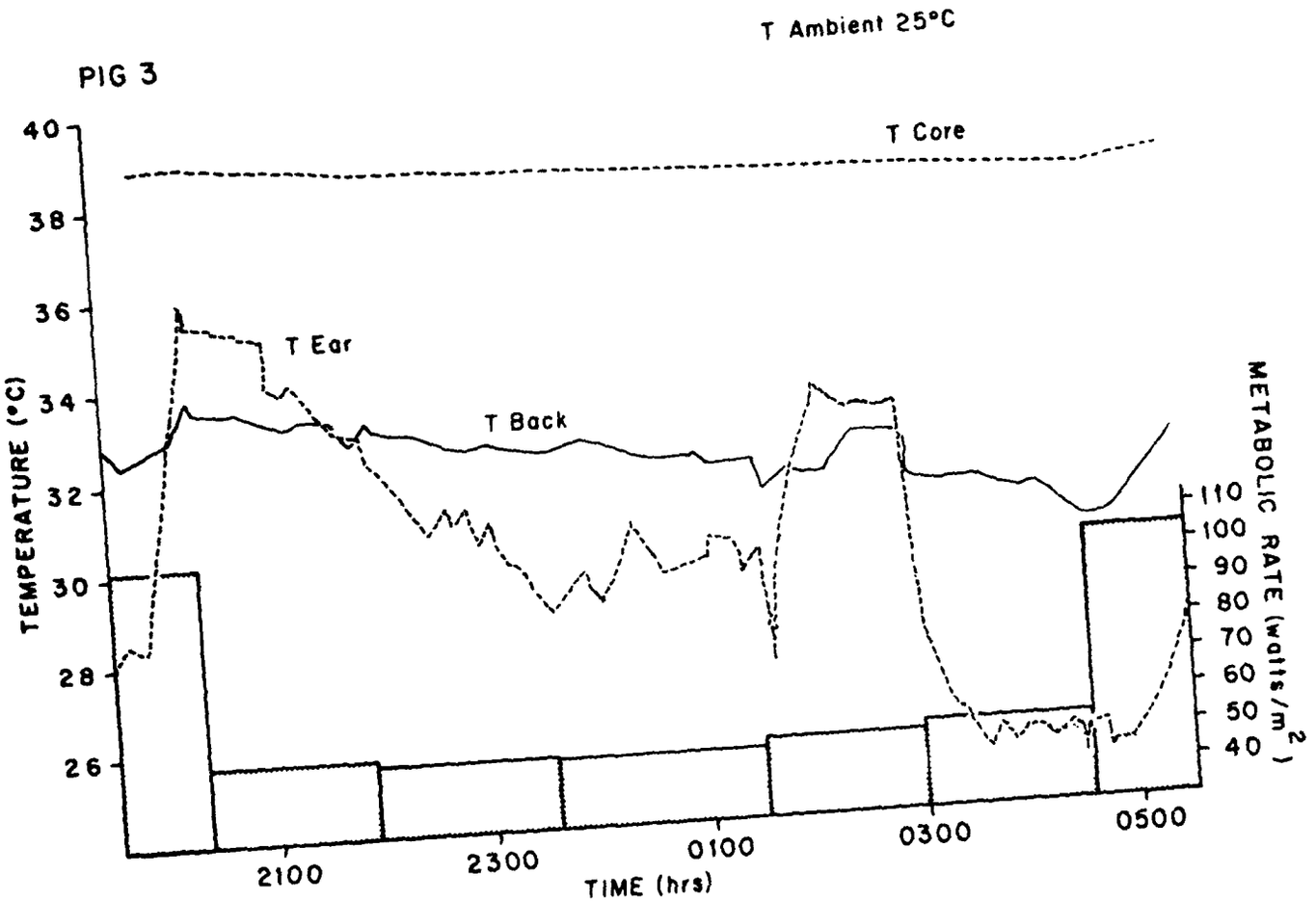

Figure 3. Variations in metabolic heat production and body temperatures of the uninjured pig during a typical overnight study within the animal's thermoneutral zone.

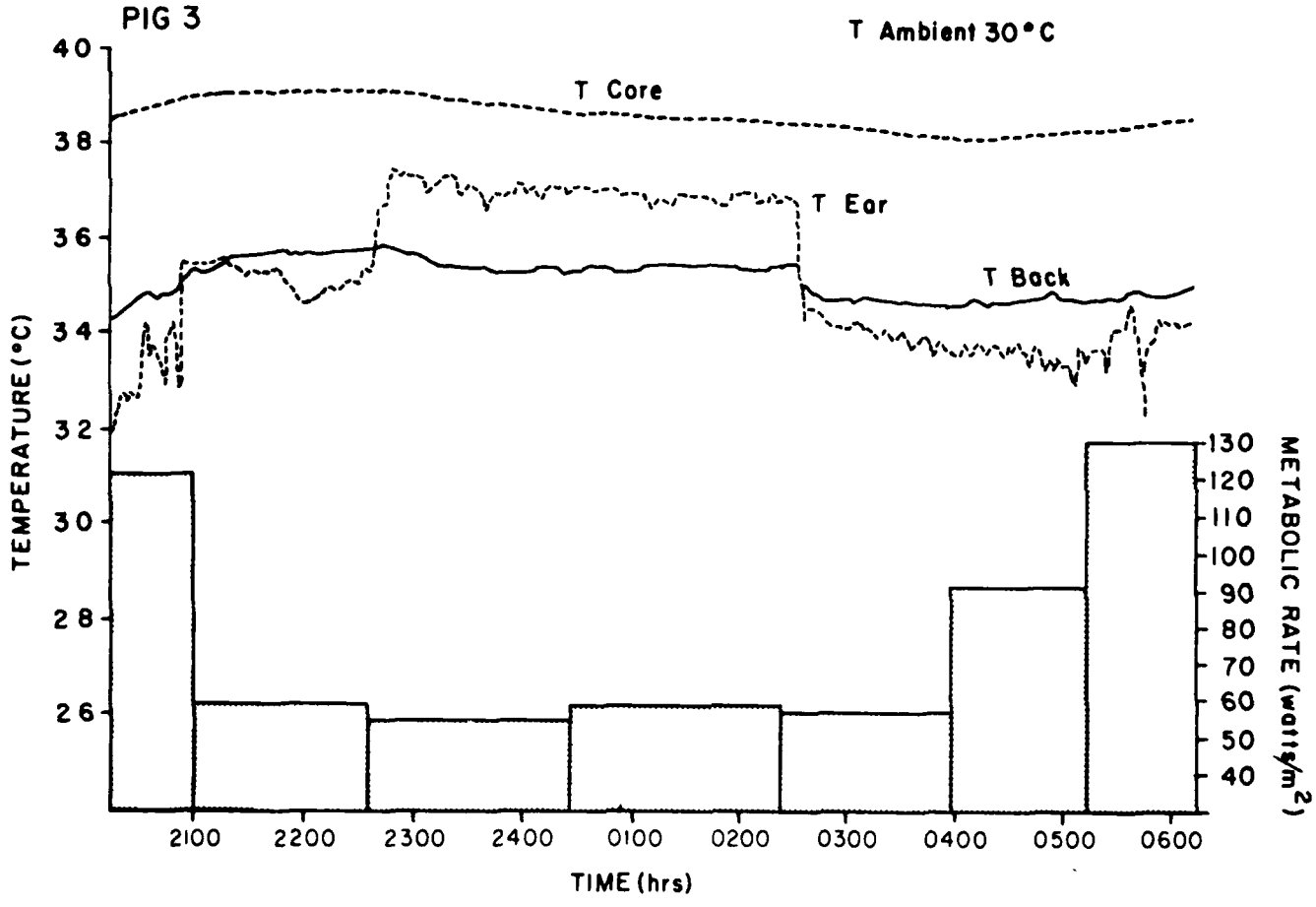

Figure 4. Variations in metabolic heat production and body temperatures of the uninjured pig during a typical overnight study in a warm but thermoneutral environment.

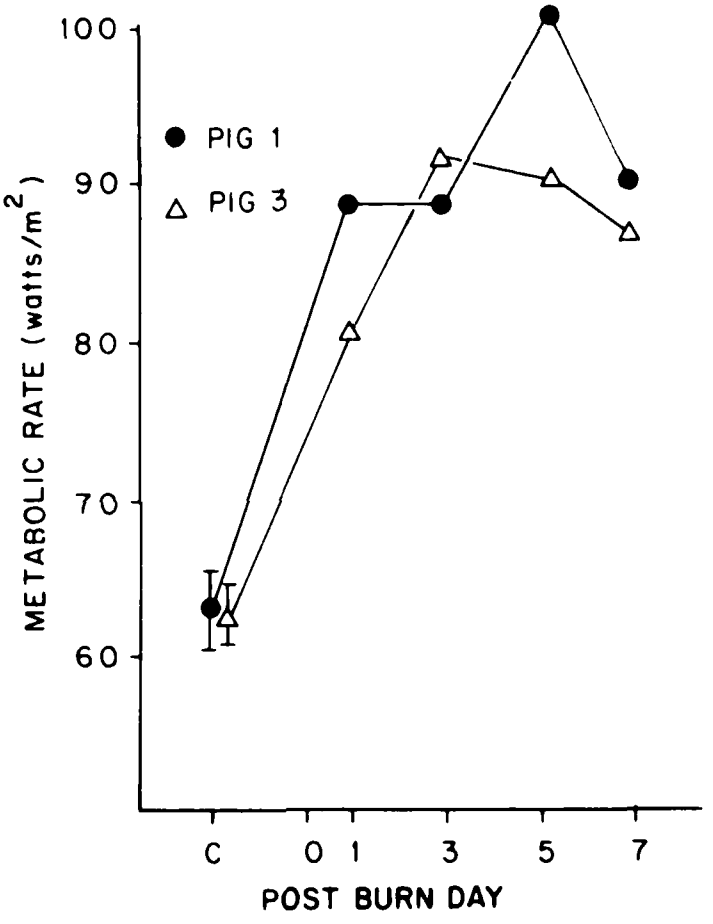

Figure 5. The increase in resting metabolic heat production of two burned pigs during the first week post injury. Chamber temperature was 25°C and the control values (C) represent the mean ± SE.

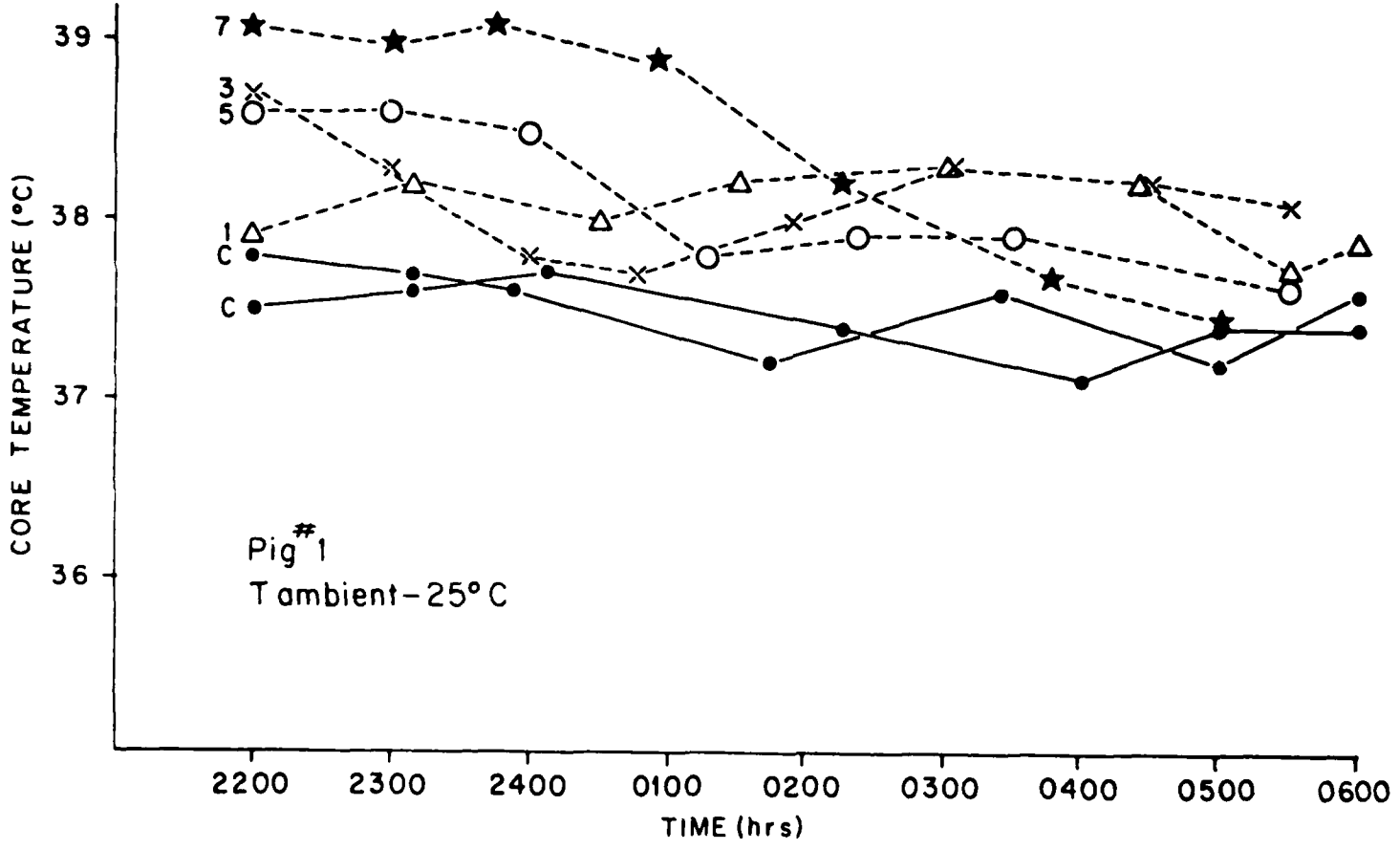

Figure 6. The gradual return of peritoneal temperatures to control levels (C) over the first week post injury. The numbers at the left refer to the postburn day studied.

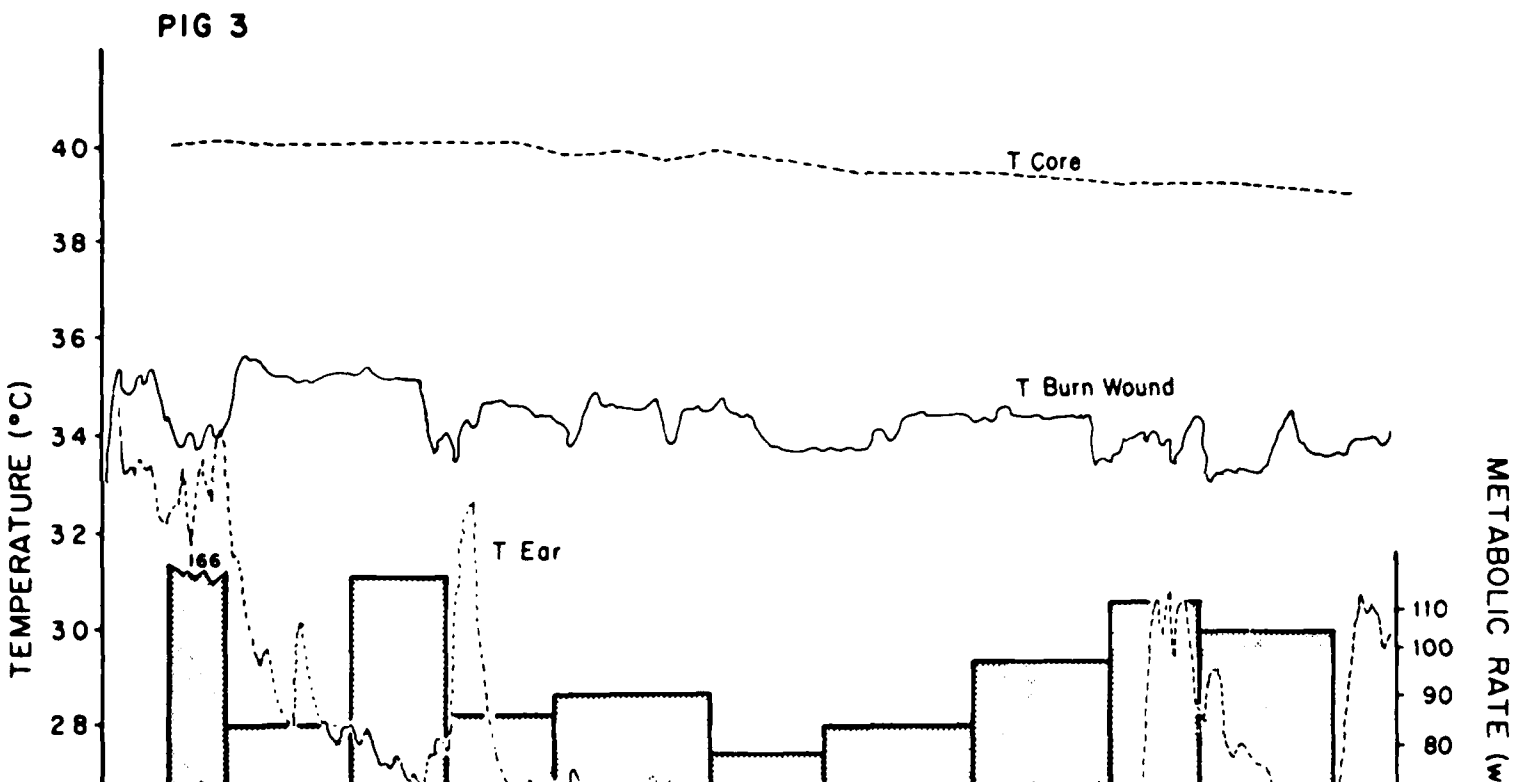

Figure 7. Variations in metabolic heat production and body temperatures of the injured pig on the third postburn day. Despite an elevated rate of heat production, the low ear temperature would suggest that the injured, febrile animal is actively reducing heat loss across uninjured skin.

DISCUSSION

Control Studies. Metabolic heat production of an uninjured pig varies with age, sex, body size, plane of nutrition, thermal environment and level of physical activity. These three female pigs were well matched for age, size and dietary intake and were studied under the same environmental conditions. To minimize the metabolic consequences of variations in physical activity, we elected to analyze only the data collected during a six-hour interval (2200-0400) each evening when the animals were usually resting quietly. Within the thermoneutral zone (25° to 30°C), the measured heat production in these three pigs was comparable to that found by others (10,11). At this level of energy turnover, oxygen consumption was 14.8 ± 0.3 ml $O_2/kg^{0.67} \cdot min$, which is within the range reported by Dauncey and Ingram (10) in overnight studies. Likewise, when the average metabolic rate of these one-year-old females is expressed in kcal/kg • day, it is consistent with the resting values reported by Brody (11) (Figure 8). This not only suggests that our indirect measurements provide valid estimates of metabolic heat production but also indicates that these animals were resting during the selected test interval.

Heat production rose sharply when these animals were subjected to ambient temperatures below 25°C (Figure 1). The ambient temperature, where the pig must increase heat production to maintain body temperature, is called the lower critical temperature (LCT). At the LCT, the animal has reached its maximal insulative capacity and the slope of the line describing the change in metabolism for a given change in ambient temperature defines the thermal conductance (reciprocal of insulation) between the animal and its environment. An LCT of 25°C and the conductance manifest by the uninjured pigs are similar to the findings of others (12,13).

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10. Dauncey MJ, and Ingram DL: Effects of dietary composition and cold exposure on non-shivering thermogenesis in young pigs and its alteration by the β-blocker propranolol. Br J Nutr 41:361, 1979.

11. Brody S: Bioenergetics and Growth. New York: Reinhold Publishing Corp., 1945, p 466.

12. Close WH, and Mount LE: The rate of heat loss during fasting in the growing pig. Br J Nutr 34:279, 1975.

13. Mount LE: Adaptation to thermal environments - Man and his productive animals. Baltimore: University Park Press, 1979, pp 190-192.

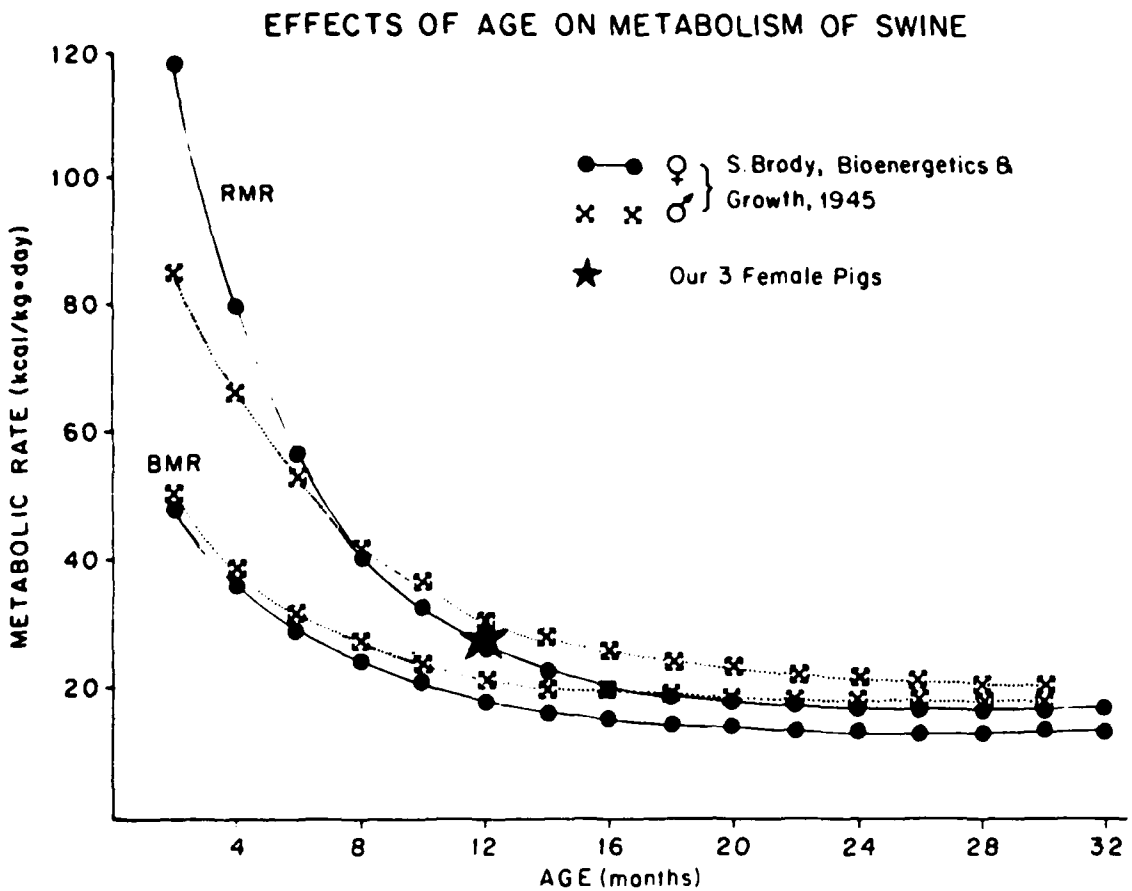

Figure 8. The resting metabolic rate (RMR) of our one-year-old female pigs is slightly above basal metabolic rate (BMR) and consistent with that reported by others.

Burn Studies. Heat production was elevated in both animals by the first day post-injury and remained 30-40 percent above resting levels throughout the one-week period of observation. "Ebb phase" hypometabolism was, therefore, not evident in either pig. The hypermetabolic response of these pigs is less than the 40-50 percent increase reported for patients with the same size injury (1, 14) but is comparable to the 20-40 percent increase seen in goats (15). Rats with this size burn have a more limited metabolic response and most, if not all, of the extra metabolism can be eliminated by increasing ambient temperature (6-8).

Associated with the increase in energy turnover was a transient rise in body temperatures. The initial changes in surface and core temperatures are consistent with normal thermoregulatory adjustments to a fever drive. For, despite a rise in heat production, ear pinna temperature remained near ambient levels, suggesting that the pig was vasoconstricting normal skin to conserve body heat and raise internal temperature (Figure 7). While both animals remained hypermetabolic, they became normothermic by the end of the first week. At this point, wound temperature was above that of uninjured back skin in the control animals while ear temperature either remained depressed (Pig 3) or was elevated (Pig 1). This suggests that heat loss is increased across the back wound and that blood flow to the ear was being regulated to retard (Pig 3) or promote (Pig 1) the restoration of normal core temperature. Over the next two weeks, these adjustments in heat production and body temperatures will indicate whether the injured pig must vasoconstrict normal skin to offset the accelerated heat loss across the wound or if it vasodilates to facilitate the removal of some of the extra metabolic heat being produced.

A burn goat model was hypermetabolic but also had no recognizable fever response (15). While comparable burn patients are usually febrile throughout most of the "flow phase," here are two different large animal models where fever and hypermetabolism may not be interdependent. This apparent disassociation between fever and postburn hypermetabolism deserves more attention, since it brings into question the role of endogenous pyrogens (EP) in the mediation of the hypermetabolic response to thermal injury. Pyrogens have been

14. Wilmore DW, Long JW, and Mason AD Jr: Catecholamines: Mediator of the hypermetabolic response to thermal injury. Ann Surg 180:653, 1974.

15. Aulick LH, Baze WB, Johnson AA, Wilmore DW, and Mason AD Jr: A large animal model of burn hypermetabolism. J Surg Res 31:281, 1981.

identified in serum of burn patients (16) and may be present in the febrile pig, but in these two pigs, it appears that EP may not be a basic circulating mediator of the hypermetabolism. Another interesting feature of this apparent disassociation between fever and hypermetabolism is that it would present yet another argument against the hypothesis that the extra energy turnover after burn injury is somehow the result of an elevated body temperature (1).

In the next two weeks (8-21 days postburn), we will attempt to identify just how the animal's thermoregulatory demands affect the hypermetabolic response. The first issue to address is whether injured animals are hypermetabolic because they are cold. If so, raising ambient temperatures should eliminate the hypermetabolism. If not, the metabolic response to environmental cooling will determine if this injury alters thermal sensitivity. If the increased metabolic rate is without a thermal basis, then the extra heat produced may make the animal more tolerant to external cooling. If, on the other hand, the patient becomes more sensitive to cooling, then such altered thermal drives may explain a major portion of the metabolic response to injury. In conclusion, the initial results indicated that 1) the control data were consistent with the literature, 2) the thermally injured pig became hypermetabolic and febrile, and 3) the hypermetabolic response continued while the fever abated by the end of the first week.

16. Wilmore DW: Studies of the effects of variations of temperature and humidity on energy demands of the burned soldier in a controlled metabolic room. In Annual Progress Report, U.S. Army Institute of Surgical Research 1 July 1975 - 30 June 1976, p 251.

PRESENTATIONS/PUBLICATIONS

PRESENTATIONS -

Aulick LH: A new system for long-term indirect calorimetry in unrestrained large animals. Sixth International Congress on Burns, International Society for Burn Injuries. San Francisco, California, 2 September 1982.

ANNUAL PROGRESS REPORT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

REPORT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS ON BURN INJURY IN SOLDIERS - COMPUTERIZED APPROACH TO NUTRITIONAL ASSESSMENT OF CRITICALLY ILL PATIENTS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1981 - 30 September 1982

Investigators:

Nancy K. McLaurin, R.D., Captain, AMSC
Cleon W. Goodwin, Jr., M.D.
Edwin W. Hander, B.S., M.A.

Reports Control Symbol MEDDH-288 (R1)

Unclassified

ABSTRACT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

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Reports Control Symbol MEDDH-288 (R1)

At the Institute of Surgical Research a computerized nutritional support program has been developed to aid in the computation of nutritional requirements of critically ill patients and in the ongoing evaluation of the adequacy of nutritional therapy in meeting these requirements.

The system consists of a series of computer programs and data files, and runs on a Digital Equipment Corporation model PDP-11/70 computer system with 512K bytes of main memory using RSX-11 M+ operating system. All programs run in an interactive manner on VT 100 video terminals located in tne patient care areas.

The initial dietary assessment of nutritional requirements is done on all patients shortly after admission using a series of formulas stored in the computer.

Routine dietary assessment of intake is carried out daily on selected patients using a calorie count program. The information needed to complete these dietary assessments is compiled by two dietetic assistants who weigh all the foods before and after meals, and by the nursing staff who record all the fluids received by the patients. The dietitian gathers all the necessary information and enters it in the computer. The calorie count program provides the user with daily total intake of calories, protein, fat, carbohydrate, and most minerals and vitamins.

Computer
Nutritional assessment
Critically ill

A nutrition summary program, which displays all past and current dietary data per patient, is also available. This summary provides both caloric and protein intake (other nutrients available on request) which is described in terms of the route of administration, percent predicted requirements, and nitrogen:calorie ratio. Changes in body weight are also displayed. A hard copy print-out of the summary is filed in the patient's chart. A graphic display of the nutritional balance and body weight is posted at each patient's bedside.

This nutritional support program assesses most of the nutritional parameters in one self-contained program. With the realization of the importance of nutrition for the recovery of the critically injured, this efficient, accurate and expeditious system has focused attention on the nutritional aspects of patient care and has proven to be beneficial and perhaps essential for the adequate evaluation and assessment of the critically injured.

PUBLICATIONS/PRESENTATIONS:

McLaurin NK: Computerized Approach to Nutritional Assessment of Critically Ill Patients. Presented at the 65th Annual Meeting of The American Dietetic Association, San Antonio, Texas, 20 Oct 82

ANNUAL PROGRESS REPORT

PROJECT NO.: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF BURN INJURY IN SOLDIERS - LIPOLYTIC ACTIVITY OF ADIPOCYTES FROM THERMALLY INJURED PATIENTS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1981 - 30 September 1982

Investigators:

David R. Strome, Ph.D., Captain MSC
Cleon W. Goodwin, Jr., M.D.
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO: 3M161102BS10-00, BASIC RESEARCH

REPORT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF BURN INJURY IN SOLDIERS - LIPOLYTIC ACTIVITY OF ADIPOCYTES FROM THERMALLY INJURED PATIENTS

US Army Institute of Surgical Research, Brooke Army Medical Center
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Period covered in this report: 1 October 1981 - 30 September 1982

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Reports Control Symbol MEDDH-288(R1)

Preliminary work in this laboratory has shown that it is possible to isolate viable adipocytes from human fat with our present techniques. However, the amount of fat which can be obtained by needle biopsy has proven in subsequent tests to be too small to give reproducible results with reasonable error limits. Because of this factor, the method of tissue biopsy was altered in the experimental protocol to provide larger tissue samples. Further progress on this experimental series awaits approval of the new biopsy technique for use in humans.

Hypermetabolism
Adipocyte

ANNUAL PROGRESS REPORT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

REPORT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS ON BURN INJURY IN SOLDIERS - USE OF VITAMIN SUPPLEMENTS ON BURNED PATIENTS: A NATIONAL SURVEY

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1981 - 30 September 1982

Investigators:

Nancy K. McLaurin, R.D., Captain, AMSC
Cleon W. Goodwin, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT No. 3M161102BS10-00, BASIC RESEARCH

REPORT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS ON BURN INJURY IN SOLDIERS - USE OF VITAMIN SUPPLEMENTS ON BURNED PATIENTS: A NATIONAL SURVEY

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1981 - 30 September 1982

Investigators: Nancy K. McLaurin, R.D., Captain, AMSC
Cleon W. Goodwin, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

Vitamin losses and requirements in critically ill burned patients remain undefined in the current research and clinical literature. A survey was conducted to determine what vitamin supplementation is routinely prescribed for burned patients, the amount most commonly prescribed, and the criteria for such prescriptions. The variety of responses received supports our original hypothesis that further research is needed to provide standard information and guidelines for the amounts and kinds of vitamin supplements needed for patients with large thermal injuries.

Vitamin supplementation
Critically ill patients

USE OF VITAMIN SUPPLEMENTS ON BURNED PATIENTS: A NATIONAL SURVEY

The nutritional requirements imposed on a burned patient depend primarily on the preburn nutritional status and the extent of the injury. Although the alterations in energy and protein requirements in critically ill patients have been widely researched (1-4), the impact of stress on requirements for vitamins, minerals and electrolytes is not adequately defined in this group of patients (4,5).

Thiamine, riboflavin and niacin, as components of essential enzymes and coenzymes, are needed for the catabolism of carbohydrate, fat and protein, for the generation of metabolic energy, as well as for synthesis and maintenance of tissue protein (6-8). Accordingly, the requirement for these vitamins increases in proportion to the energy requirements. Unfortunately, other vitamins do not share the same characteristic. The need for vitamin C, for instance, remains jumbled in the controversy about its requirements in both health and disease (9). The only essential function that has been elucidated in relation to vitamin C in stress and wound healing is the hydroxylation of proline and lysine during collagen synthesis (9-12).

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2. Border JR: Acute protein malnutrition in burned patients. J Trauma 19 (Suppl 11): 902, 1979

3. Blackburn GL, Bristrian BR: Nutritional care of the injured and/or septic patient. Surg Clin North Am 56: 1195, 1976

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11. Murad S, Grove D, Lindberg KA, Reynolds G, Sivarajah A, Pinnell SR: Regulation of collagen synthesis by ascorbic acid. Proc Natl Acad Sci USA 78: 2879, 1981

12. Kramer GM, Fillios LC, Bowler EC: Ascorbic acid treatment on early collagen production and wound healing in the guinea pig. J Periodontol 50: 189, 1979

Still, the precise vitamin C dosage needed for optimum wound healing is unknown (13). This same uncertainty applies to other vitamins as well. Are the absorption and metabolism of vitamins impaired due to stress? Does stress inflict an additional vitamin loss from the body? Does vitamin supplementation benefit burn patients and what is the proper dosage? These questions currently remain unanswered.

This paper describes the use of vitamin supplements in burned patients in the United States. The information was gathered by a questionnaire (Figure 1) to determine the vitamin supplements routinely prescribed for burned patients, the amount most commonly prescribed, and the criteria for such prescriptions. The results of this survey support our hypothesis that further research is needed to provide standard information on guidelines for the amounts and kinds of vitamin supplements needed for thermally injured patients.

MATERIAL AND METHODS

The questionnaire (Figure 1) was sent to 134 physicians and 137 dietitians who provide direct care to burn patients in 137 hospitals throughout the United States. The hospitals selected were those listed in the Burn Care Services Roster distributed by the American Burn Association in 1980.

RESULTS

Forty-seven percent of the questionnaires were completed and returned, two percent were undeliverable, and the rest did not reply.

Eighty-seven percent of the responders used vitamin supplementation as a routine for all burned patients admitted to their treatment facilities. The remaining 13 percent of the questionnaires indicated vitamin supplementation based mainly on burn size, nutritional status prior to admission, and patients' spontaneous intake (Table 1). Ninety-seven percent of those that prescribe vitamins routinely use a full spectrum multivitamin preparation. The rest used individual vitamin preparations for thiamine, riboflavin, niacin, ascorbic acid, vitamin K, and vitamin B-12 supplementation.

Many of the full spectrum multivitamin preparation users also prescribe additional individual vitamins. For instance, 72 percent prescribe additional vitamin C (Tables 2 and 3). Several vitamins were supplemented as needed based on patients' histories and physical symptoms. Examples of these are thiamine, which is

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13. Vitamin C, disease and surgical trauma. Br Med J 1: 437, 1979

supplemented for alcoholism in ten percent of the questionnaires, and vitamin K, which is supplemented for bleeding problems in eight percent of the questionnaires. Other vitamins occasionally added to supplement the multivitamin preparations were folic acid, vitamin B-12, vitamin A, vitamin D, vitamin E, pyridoxine, and pantothenic acid.

Of those who use multivitamin preparations, 37 percent provide 100 percent of the Recommended Dietary Allowances, 1980. Five percent provide less, and 58 percent provide more than the Recommended Dietary Allowance.

Although the questionnaire did not ask specifically about mineral supplementation, several participants specified their usage as follows: zinc - 24 percent, iron - seven percent, copper - one percent. These levels of usage are not definitive, since many participants may not have mentioned the mineral preparations routinely employed in their facilities.

DISCUSSION

The results of the survey show that guidelines for vitamin supplementation in burned patients are nonexistent. Most health care providers appear to feel that vitamin supplementation is important during stress and that multivitamin preparations are the best way of supplying these vitamins. Yet, only 58 percent of the multivitamin users provide more than 100 percent of the normal Recommended Dietary Allowance. Administration of vitamins in excess of the RDA level may be justified by the empiric observation that increased caloric requirements necessitate elevated vitamin requirements. On the other hand, those providers who do not provide more than 100 percent of the RDA may feel that the patient will get the necessary additional vitamins from the enteral nutritional support prescribed.

Vitamin C is a reducing agent responsible for the activation of several enzymes and their cofactors (14). It also affects the immune response system by facilitating leukocyte mobility (14,15) and wound healing by the activation of prolyl hydroxylase and the subsequent hydroxylation of peptide-bound proline by the activated enzyme (16). Vitamin C has a physiological role in the detoxification of histamine, which is believed to explain the beneficial effect of vitamin C observed in various stress conditions (16). The unanswered question is how much should be

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14. Vitter RW: Nutritional aspects of ascorbic acid: uses and abuses. West J Med 133: 485, 1980

15. Ascorbic acid: Immunological effects and hazards. Lancet 1: 308, 1979

16. Chatterjee IB: Ascorbic acid metabolism. World Rev Nutr Diet 30: 69, 1978

supplemented. Are megadoses[1] better than physiological doses (14)? Fifty-six percent of the respondents who provide more than 100 percent of the RDA by multivitamin preparations, supplemented this vitamin intake by additional vitamin C.

Some vitamins, particularly vitamins A and D, are known to be toxic in large amounts. On the other hand, water soluble vitamins are generally considered harmless even in larger doses. However, when the intake of vitamin C increased in popularity as a "cold remedy", so did the number of studies addressing ascorbic acid's benefits and hazards. Megadoses of vitamin C have been implicated in the formation of calcium oxalate and urate urinary calculi, decreased vitamin B-12 availability, hypovitaminosis C after withdrawal, diarrhea, enhancement of metal toxicity, potentiation of aspirin-induced mucosal ulceration, false-negative guaiac occult blood tests, and interference with tests for glucosuria (6,14,15,17,18). Yet, because patients with normal renal function can tolerate exceptionally high doses of vitamin C without having calcium oxalate deposition in their kidneys and because the gastrointestinal tract symptoms are usually reversible upon discontinuance of vitamin C, some consider vitamin C nontoxic (15,19,20).

Niacin in large amounts (3-10 grams per day) may cause flushing, abnormal liver function, vascular changes, hyperuricemia, dryness of the skin, nausea, diarrhea, abdominal pain, and glucose intolerance (6). Many times, some of the gastrointestinal problems of burned patients (diarrhea, nausea, vomiting) are attributed to antacids, antibiotics or tube feeding administration while many of these symptoms in fact could be due to megadoses of some vitamins. Evaluation of such symptoms should include consideration of the effects of all medications, including vitamin preparations.

CONCLUSIONS AND IMPLICATIONS

The variety of responses received in the survey supports our original hypothesis that further research is needed to provide standard information on guidelines for the amounts and kinds of

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[1]Unless otherwise specified, megadoses refers to dosages higher than the Recommended Dietary Allowances.

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17. Nagengast FM: Vitamin C and guaiac occult blood test. Lancet 1: 614, 1981
18. Hoyt CJ: Diarrhea from vitamin C. JAMA 244: 1674, 1980
19. White JD: No ill effects of high-dose vitamin C. N Engl J Med 304: 1491, 1981
20. Hoffer A: Ascorbic acid and toxicity. N Engl J Med 285: 635, 1971

vitamin supplements needed for burned patients. Because of the cost of vitamin supplements and the possible dangers of toxicity from megadoses, the need for further defining the benefits of vitamin dosages above the Recommended Dietary Allowances for the burned and other critically ill patients is imperative.

SUMMARY

Vitamin losses and requirements in critically ill and burned patients remain undefined in the current research and clinical literature. A survey was conducted to determine the vitamin supplementations routinely prescribed for burned patients, their dosages, and the criteria for administration. The questionnaire was sent to 271 health care providers (dietitians and physicians) who work in burn care facilities in the United States. Forty-seven percent of the total questionnaires were completed and returned. Of that group, 82 percent of the respondents routinely prescribed vitamin supplementation. Most (97%) who prescribed routine vitamin supplementation use some kind of multivitamin preparation. Fifty-eight percent of the multivitamin dosages exceeded 100 percent of the Recommended Dietary Allowance, 1980. Several respondents pointed out that extra vitamins are given in addition to the multivitamin preparations. Vitamin C is given in 72 percent of the questionnaires in addition to the multivitamin preparations. Most facilities use more than one specific criterion to prescribe vitamins when supplementation is not routine. Burn size, nutritional status prior to admission, and poor dietary intake were the criteria most commonly identified. Further research is needed to provide guidelines for the amount and kind of vitamin supplements needed for burned patients.

PRESENTATIONS

McLaurin NK: Use of Vitamin Supplements on Burned Patients: A National Survey. Presented at 65th Meeting of the American Dietetic Association, Poster Session, San Antonio, Texas, 20 October 1982

Figure 1: Questionnaire used in the survey

QUESTIONNAIRE

1. Are vitamins prescribed routinely for the burn patients?

Yes_____ No_____

2. If yes, please complete:

	Amount	How Often (Daily, weekly, twice a week)
Full spectrum multivitamin preparations	_____	_____
Thiamine	_____	_____
Riboflavin	_____	_____
Niacin	_____	_____
Vitamin C	_____	_____
Vitamin K	_____	_____
Vitamin B_{12}	_____	_____
Folic Acid	_____	_____
Other		

3. If multivitamins are used, what is their composition?

Provides 100% RDA* _____

Provides more than 100% RDA _____

Provides less than 100% RDA _____

4. If vitamins are not prescribed routinely, in which instances are they used, if any ?

_____

_____

*Recommended Dietary Allowances, 1980

Table 1: Criteria for vitamin supplementation when prescription is not routine

Criteria	No. of Respondents*
Large burn size	5
Nutritional status prior to admission	5
Poor intake	5
Dietitian's advice	4
Physician's discretion	3
Patient's age (pediatric and elderly)	1
Patient's request	1
Nutritional support provided by total parenteral nutrition only	1
Deficiencies indicated by laboratory tests	1
Poor wound healing	1

*Most facilities use more than one specific criterion

Table 2. Vitamins prescribed in addition to the full spectrum multivitamin preparation

Vitamin	Percent of Respondents
Ascorbic acid	72
Thiamine	9
Niacin	3
Riboflavin	2

Table 3. Dosage of supplemental Vitamin C (# of users - 77)

Milligrams Per Day	Percent of Respondents
< 1000	25
1000	31
1500	23
2000	17
3000	3
unknown	1

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0G 1842	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8A. DISP'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. CHANGE	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BB	304
b. CONTRIBUTING				
c. XXXXXXXXX	STOG 80-7.2:5			

11. TITLE (Procede with Security Classification Code)*

(U) Assessment of L-Triiodothyronine Therapy in Thermally Injured Patients

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 08	Cont	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	1.2	110
	CURRENT 83	1.2	60

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Institute of Surgical Research

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RESPONSIBLE INDIVIDUAL

NAME: Basil A. Pruitt, Jr., MD, COL, MC

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME: POC: DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) L-Triiodothyronine; (U) Therapy; (U) Burn Patients; (U) Hypothyroidism

23. TECHNICAL OBJECTIVE.* 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with security Classification Code.)

23. (U) To assess the potential benefit of treatment with thyroid hormones in burned soldiers.

24. (U) Characterize the nature, extent, and significance of altered thyroid economy in burn patients and determine changes in moribidity, mortality, and circulating hormones after replacement therapy with thyroid hormones.

25. (U) 8110 - 8209. Based on suppression of serum T_3 in nearly all burn patients, our initial randomized clinical trial treating all patients throughout their course was performed and revealed no harmful or beneficial effects of T_3 treatment. However, our results from seven separate thyroid-related assays in untreated patients indicate very complex alterations of thyroid hormone economy, including changes in transport binding, peripheral conversion of T_4, thyroid hormone feedback on TSH secretion, and production and elimination rates of T_3 and T_4. Multiple regression and covariance analyses indicated less suppression of free T_4 and T_3 than indicated by the respective free index. Less elevation of in vitro T_3 uptake than of dialysable fraction of T_4 and of T_3 in burns indicates the presence of binding inhibitor(s) that may react not only with endogenous carrier protein but also with in vitro solid matrix. Severe depletion of T_4 usually occurs before

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

death in nonsurvivors. After further characterizing thyroid hormone economy (binding, kinetics) in these patients and identifying clinical markers for the T_4 depletion state, preferably advance markers (in progress), the proper patients can be selected for a trial of T_4 therapy and the proper variables can be followed to asess its effect.

ANNUAL PROGRESS REPORT

PROJECT NO.: 3M161102BS10-00, BASIC RESEARCH

REPORT TITLE: ASSESSMENT OF L-TRIIODOTHYRONINE THERAPY IN THERMALLY INJURED PATIENTS--THE HYPERMETABOLIC LOW TRIIODOTHYRONINE SYNDROME IN THERMALLY INJURED PATIENTS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1981 - 30 September 1982

Investigators:

George M. Vaughan, Major, MC
Leonard G. Seraile
William F. McManus, Colonel, MC
Basil A. Pruitt, Jr., Colonel, MC
Arthur D. Mason, Jr., M.D.

Reports Control symbol MEDDH-288(R1)
UNCLASSIFIED

ABSTRACT

PROJECT NO.: 3M161102BS10-00, BASIC RESEARCH

REPORT TITLE: ASSESSMENT OF L-TRIIODOTHYRONINE THERAPY IN THERMALLY INJURED PATIENTS--THE HYPERMETABOLIC LOW TRIIODOTHYRONINE SYNDROME IN THERMALLY INJURED PATIENTS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1981 to 30 September 1982

Investigators: George M. Vaughan, Major, MC
Leonard G. Seraile
William F. McManus, Colonel, MC
Basil A. Pruitt, Jr., Colonel, MC
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

Burned soldiers (and, indeed, many other types of ill patients) have low circulating levels of thyroid hormones, triiodothyronine (T_3) and tetraiodothyronine (T_4). It is clear that any treatment of burned soldiers with thyroid hormones will be extremely difficult to apply and evaluate rationally unless we obtain a better understanding of how these hormones are handled in these patients and how this relates to control of endogenous thyroid hormone production and the effects of these hormones on critical functions such as general metabolic rate and defense against sepsis. There is great complexity in the potential changes of thyroid hormone economy in severe illness, and these changes must be better understood in order to eventually utilize them in determining which abnormalities are adaptive (and ought not be intercepted) and which are detrimental and ought to be changed for the patient's advantage.

These concerns are at the very forefront of the present-day approach to understanding the pathophysiology leading to a patient's death. The endocrine-metabolic patterns related to thyroid economy and energy/substrate metabolism exhibit commonalities among all major types of illness (including medical, surgical, infective, general traumatic, and thermal traumatic). These patterns, though perhaps more marked in burn patients, generally appear to relate more to the severity than to the type of non-thyroidal illness (NTI) and possibly may be related to non-survival (1). What is so far lacking is a useable understanding of the specific elements of the altered hormone-metabolic pattern, such as characteristics of thyroid hormone transport, kinetics, and control by the hypothalamic-pituitary unit; action of the hormones on central nervous function, pituitary secretion, and thermogenesis; and interaction of thyroid hormones with other hormones (such as catecholamines, glucagon, insulin, growth hormone, and reproductive hormones) on thermogenesis and substrate flow. Investigation of many of these elements is underway, and the present report is concerned with thyroid hormone transport. Understanding of this and the other elements may eventually allow a selection of which patients, at which point in time, should have which hormonal abnormality corrected.

L-Triiodothyronine
Therapy
Burn Patients
Hypothyroidism

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1. Becker RA, Vaughan GM, Ziegler MG, Seraile LG, Goldfarb IW, Mansour EH, McManus WF, Pruitt BA Jr., Mason AD Jr: The hypermetabolic low triiodothyronine syndrome in burned soldiers. In US ArmyInstitute of Surgical Research Annual Research Progress Report FY 1981, Ft. Detrick, MD: US Army Medical Research and Development Command, 1981, pp 288-305.

ASSESSMENT OF L-TRIIODOTHYRONINE THERAPY IN THERMALLY INJURED PATIENTS

METHODS

Thirteen serum samples were taken from normal control individuals (mean age 34.1 years) and 21 samples from burn patients (mean age 41.4 years, mean total burn size 37.0% body surface area) at various times over the first month after injury. Total T_4 and T_3 were determined by radioimmunoassay, and the extent of binding was assessed by equilibrium dialysis and by *in vitro* T_3 charcoal uptake. The free T_4 and free T_3 respectively represent the product of total T_4 or T_3 and the dialyzable fraction (DF) of the respective total hormone; that is, free T_4 = (total T_4)(DFT_4) and free T_3 = (total T_3)(DFT_3).

The free T_4 index and free T_3 index (FT_4I and FT_3I) represent the product of the respective total hormone concentration and the *in vitro* T_3 uptake (T_3U). The T_3U is traditionally expressed as the ratio of ^{125}I-tracer-T_3 in the matrix (charcoal) to the total ^{125}I-tracer-T_3 after incubation. However, it is now considered that expression of the T_3U as the ratio of ^{125}I-tracer T_3 in the matrix to that in the serum after incubation better represents the propensity of T_4 or T_3 to be unbound by plasma transport proteins. Using this corrected T_3U (T_3UC) then gives corrected free indices, FT_4IC and FT_3IC, as the products of total T_4 or T_3 respectively and the T_3UC. Table 1 summarizes the basic features determining these values.

TABLE 1. DETERMINATION OF CORRECTED FREE THYROID HORMONE INDICES*

Uncorrected	Corrected
$T_3U = \frac{\text{matrix tracer-}T_3}{\text{total tracer-}T_3}$	$T_3UC = \frac{\text{matrix tracer-}T_3}{\text{serum tracer-}T_3}$
$FT_4I = \left(\frac{T_3U}{0.3}\right)(T_4)$	$FT_4IC = \left(\frac{T_3UC}{0.3/0.7}\right)(T_4)$
$FT_3I = \left(\frac{T_3U}{0.3}\right)(T_3)$	$FT_3IC = \left(\frac{T_3UC}{0.3/0.7}\right)(T_3)$

*(T_4) and T_3) represent respective total concentrations. The denominators (0.3 and 0.3/0.7) represent a normal calibrator sample provided in the T_3U kit, which makes the final result (index) to be essentially a corrected T_4 and T_3 value exhibiting ranges of values resembling those of the original T_4 and T_3 values respectively. Of course, the indices (FT_4I, FT_3I, FT_4IC, FT_3IC) have no unit designations.

RESULTS

Tables 2 and 3 show the results that illustrate the problems in thyroid hormone transport binding in burn patients.

TABLE 2. DIALYSIS OF THYROID HORMONES

	DFT_4	free T_4 ng/dl	DFT_3	free T_3 pg/dl
Controls				
mean	.0002815	2.135	.002271	315.7
S.E.	.0000055	0.103	.000081	11.6
Burns				
mean	.0003757*	1.120*	.003733*	177.1*
S.E.	.0000092	0.079	.000156	15.4

*$p < 0.001$ vs controls (Student t test).

TABLE 3. CORRECTED T_3U AND FREE THYROID HORMONE INDICES.

	T_3UC	FT_4IC	FT_3IC
Controls			
mean	0.3725	6.522	121.0
S.E.	0.0133	0.247	4.8
Burns			
mean	0.4548*	3.199*	52.5*
S.E.	0.0175	0.243	5.6

*$p < 0.001$ vs controls (Student t Test).

DFT_4 (1.34-fold) and DFT_3 (1.64-fold) were highly significantly elevated in burns compared to controls, and free T_4 (to 52.5% of control) and free T_3 (to 56.1%) were highly significantly suppressed in burns. T_3UC (1.22-fold) was highly significantly elevated, and FT_4IC (to 49.1% control) and FT_3IC (to 43.4%) were highly significantly suppressed in burns. Covariance analyses indicated that the elevation of T_3UC in burns was less than the elevation of DFT_4 and DFT_3, because in burns, the FT_4IC was suppressed to a greater extent than was the free T_4 ($p < 0.01$), and the FT_3IC was suppressed to a greater extent than was the free T_3 ($p < 0.001$).

DISCUSSION

Results from both dialysis and T_3UC-derived indices demonstrate markedly significant reduction in plasma binding for both T_4 and T_3 in the circulation of burn patients. The dialysis approach involves only the distribution of hormones across a membrane (not permeable to protein) between plasma and buffer, whereas the T_3UC approach involves actual competition between plasma and matrix for T_3. Because in the latter approach the plasma binding abnormality was less pronounced, it is suggested that a circulating inhibitor of binding (which may or may not be responsible for inhibited binding to plasma) inhibits binding to the T_3UC matrix. This would allow greater accumulation of tracer in plasma than expected from the dialysis result which is obtained without use of a competing matrix.

Thus, the thyroid hormone binding deficit in burn patients may extend beyond relevance to plasma proteins. This raises the question of whether thyroid hormones may also have defective binding to tissue sites in burn patients, which may be approached by assessing the plasma disappearance of tracer hormone from plasma in the distribution phase (compared with the elimination phase) of kinetic studies.

PRESENTATIONS/PUBLICATIONS

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0G 1843	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. CHANGE	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	075
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)* (U) Micromethod For Assessment of Serum Opsonic Capacity in The Burned Patient

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 06	Cont	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	0.6	43
FISCAL YEAR CURRENT 83	0.6	53

19. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

20. PERFORMING ORGANIZATION
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NAME: Basil A. Pruitt, Jr., MD, COL, MC
NAME: Roger W. Yurt, MAJ, MC POC: DA

22. KEYWORDS (Procede EACH with Security Classification Code) (U) PMN Leukocytes; (U) Chemiluminescence; (U) Opsonization; (U) Immunoglobulins; (U) Complement; (U) Burn Injury: (U) Human Volunteer

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) The nonspecific opsonic capacity of sera from patients following burn injury will be compared to normal control sera. Qualifications of opsonic capacity will be based upon the rate and magnitude of oxidative microbial activation as measured by amplified chemiluminescence using a set number of functional polymorphonuclear leukocytes (PMN) challenged with a set concentration of either zymosan or bacteria (Staphylococcus aureus or Pseudomonas aeruginosa). By holding zymosan and PMN leukocyte number constant, chemiluminescent activity will reflect the opsonic activity of sera. Opsonic dysfunction has been reported in severe trauma patients, and may result in increased susceptibility to infection. The present research will provide a means of monitoring immunocompetence of severely injured military patients.

24. (U) These functional measurements will be correlated with immunologic data, such as serum complement and immunoglobulin, quantified by immunoelectrophoretic and immunodiffusion techniques.

25. (U) 8110 - 8209. An improved methodology, requiring less blood and allowing measurement of both opsonic and granulocyte function on the same specimen, has been developed and is being tested at present. Twenty patients have been added to the study.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: MICROMETHOD FOR ASSESSMENT OF SERUM OPSONIC CAPACITY IN THE BURNED PATIENT

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1981 - 30 September 1982

Investigators:

Robert C. Allen, M.D., Ph.D., Major, MC
Roger W. Yurt, M.D. Major, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: MICROMETHOD FOR ASSESSMENT OF SERUM OPSONIC CAPACITY IN THE BURNED PATIENT: CHEMILUMIGENIC PROBING OF THE HUMORAL-PHAGOCYTE AXIS OF IMMUNE DEFENSE IN THE BURN INJURY PATIENT

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1981 - 30 September 1982

Investigators: Robert C. Allen, M.D., Ph.D., Major, MC
Roger W. Yurt, M.D., Major, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

The humoral-phagocyte axis of immunity provides the primary host defense against bacterial and in some cases fungal infection. Evidence suggests that both elements of this information-effector system may be defective in patients with severe burn injury. The microbicidal action of granulocytes, the phagocyte element of the axis, is effected via generation of oxygenating agents. As such, introduction of chemiluminigenic probes, high chemiluminescent quantum yield substrates susceptible to oxygenation, allows ultrasensitive measurement of stimulated phagocyte oxygenation activity based on single photon counting of the resulting luminescence. Serum opsonic capacity can also be assayed by measuring the rate of activation of control granulocytes using the probe approach. Up to the present time, fifty patients have been entered into the study. Modification of the technique for study of granulocyte oxygenation activity and a simplified method for titration of plasma opsonic activity were tested during the period covered by this report. Improvements include greater sensitivity and ease of testing which will hopefully increase the potential use of these techniques for monitoring patient populations highly susceptible to sepsis.

Burn Injury
Chemiluminescence
Chemiluminigenic probe
Complement
Dimethyl biacridinium dinitrate
Polymorphonuclear leukocyte
Granulocyte
Luminol
Opsonin
Phagocyte

MICROMETHOD FOR ASSESSMENT OF SERUM OPSONIC CAPACITY IN THE BURNED PATIENT: CHEMILUMIGENIC PROBING OF THE HUMORAL-PHAGOCYTE AXIS OF IMMUNE DEFENSE IN THE BURN INJURY PATIENT

Acute defense against infection is primarily effected via the humoral-phagocyte axis of immunity. In burn patients, the tendency for infection by opportunistic pathogens implies defective immune defense, and the empirical evidence presented support dysfunctions of both humoral mechanisms and granulocyte metabolism.[1-3]

A newly developed experimental approach employing chemiluminigenic probes for the study of the humoral-phagocyte defense system has been reported.[4-5] Probing is based upon measurement of phagocyte (granulocytes and monocytes) oxygenation activities in response to immune or chemical stimulation. Chemiluminigenic probes (CLP's), such as cyclic hydrazides and biacridinium salts, serve as high quantum yield bystander substrates yielding photons on oxygenation. As such, probing can provide a measure of cell response to stimulation. If the function and number of effector phagocytes are held constant, the CLP approach can also provide a method for titrating the functional opsonic capacity of a serum or plasma specimen with respect to a given antigen.

The results and interpretations of our initial CLP research were described in the 1981 Annual Report. The present report describes simplification and improvements in methodology that allow greater applicability as a clinical laboratory technique.

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1. Alexander JW, McClellan MA, Ogle CK, and Ogle JD: Consumptive Opsoniopathy: Possible Pathogenesis in Lethal and Opportunistic Infections. Ann Surg 1976; 184: 672-678.

2. Bjornson AB, Altemeier WA, Bjornson HS. Host Defense Against Opportunist Microorganisms Following Trauma. Ann Surg 1978; 188: 93-101.

3. Alexander JW, Wixsom D. Neutrophil Dysfunction and Sepsis in Burn Injury. Surg Gynecol Obstet 1970; 130: 431-438.

4. Allen RC. Biochemiexcitation: Chemiluminescence and the Study of Biological Oxygenation Reactions. In: Adam W. and Cilento G., eds "Chemical and Biological Generation of Excited States." New York Academic Press, 1982: 309-344.

5. Allen RC and Pruitt BA Jr.: Humoral-Phagocyte Axis of Immune Defense in Burn Patients: Chemoluminigenic Probing: Arch Surg 1982; 117: 133-140.

METHODS AND MATERIALS

Patient Data:

Twenty-six new patients were entered into the study this year. As many as thirty separate specimens were obtained per patient. The study was prospective in that daily independent clinical assessments were recorded and these data were held separate until completion of the period of laboratory testing. The data were so treated to eliminate the possibility of bias in comparing clinical and laboratory findings.

Blood specimens were obtained in the early morning at the time of routine venipuncture. In addition, samples were collected when blood cultures were drawn. To minimize patient blood loss, the specimens were collected in pediatric (approximately 2 ml capacity) blood tubes. This specimen was used for both granulocyte and humoral opsonic measurements. Informed consent was obtained from the patients and controls studied.

PREPARATION OF CLP TEST VIALS

Two chemiluminigenic probes were routinely employed in the present study. Luminol (5-amino-2,Ø3-dihydro-1, 4-phthalazinedione) and DBA (lucigenin; 10,Ø10'-dimethyl-9, 9-biacridinium dinitrate) were purchased from Aldrich Chemical Company. The probes were directly prepared in veronal (barbital) buffered saline pH 7.2 containing Ca^{++}, Mg^{++}, plus albumin (0.1% w/u) and glucose (0.1% w/u)[6]. After combining all of the reagents, the final concentration was 10 µM for the luminol vials and 50 µM for the DBA vials. The luminol vials also contained dimethyl sulfoxide (DMSO) at a final concentration of approximately 1 volume DMSO per 1000 volumes buffer. The DBA vials did not contain DMSO.

The vials were prepared prior to beginning the experiments. The concentrations of components in the bulk preparations were tested by measurement of absorbance (extinction coefficient). The CLP buffer was then aliquoted to the sterile siliconized vials, and the vials were frozen at -70°C until used.

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6. Mayer MM: Complement and Complement Fixation. In: Kabat EA, ed "Experimental Immunochemistry", Springfield, Charles C. Thomas Publ., 1971: 133-162.

PREPARATION OF STIMULI

The stimuli, opsonified zymosan (OpZy) and phorbol-12-myristate-13-acetate (PMA) were prepared as previously described.[5]

GRANULOCYTE OXYGENATION MEASUREMENTS

Whole blood (approximately 2 ml) was collected in pediatric EDTA tubes. Aliquots were removed for Coulter counting and preparation of differential slides. A 50 µl aliquot of well mixed whole blood was diluted 1 to 100 with phosphate buffered saline (PBS) pH 7.2, and further mixed on a tilt table.

Fifty microliters of the diluted specimen (equivalent to 0.5 µl whole blood) were added to the prepared CLP test vials at room temp (23°C). The vials were placed in the single photon counter and measured for 30 minutes to obtain background chemiluminescence before adding either OpZy or PMA to initiate stimulation. Poststimulation chemiluminesence was measured over a 90 minute period.

PLASMA OPSONIC CAPACITY MEASUREMENTS

After removing aliquots of whole blood for white blood count, differential, and CLP measurement, the remaining blood specimen was centrifuged and the plasma removed. The plasma was frozen at -70°C until tested. Opsonic capacity was tested as previously described except that control whole blood (0.5 µl equivalent) was used as the source of phagocytes instead of isolated granulocytes.[5]

SINGLE PHOTON COUNTING

Single photon counting was as previously described[5].

RESULTS AND DISCUSSION

Granulocyte oxygenation activities were differentially measured using 0.5 µl of whole blood as the source of phagocytes. Luminol and DBA were employed as the CLP's. When luminol was used, immune (OpZy) and chemical (PMA) stimuli were tested. Only PMA was used as stimulus with DBA as the CLP.

The present technique differs from the previously described method in that the amount of whole blood tested has been decreased by a factor of 20. Using 0.5 µl of whole blood in a final volume of 2.0 ml effectively eliminates the problem of hemoglobin quenching of the emitted luminescence.[7]

In order to increase the sensitivity of measurement, and also drive the reaction toward a zero-order condition with respect to CLP, the concentrations of luminol and DBA were increased 20 fold and 100 fold respectively. The CLP's were not found to be cytotoxic at these concentrations.

The patient's blood was analyzed daily. The results of a single analysis are presented in Figure 1. Each of the three different measurements were done in triplicate. Note that the CLP responses differ in temporal kinetics with respect to the combination of CLP and stimulus tested. These differences reflect the differential measurement of oxygenation activities with reference to the location and type of oxygenating agent measured. Figure 2 presents the same data plotted as the cumulative or integral photon count with respect to time.

The advantages of this modified method are: (1) decrease in the amount of whole blood required for testing and therefore a decrease in the effect of plasma introduced with the whole blood, (2) decrease in hemoglobin absorption of the emitted light, (3) decrease in the amount of blood required for testing, and (4) increase in the sensitivity of measurement.

The opsonic capacity of plasma was also tested by a modification of the previously reported technique[5]. Instead of using isolated, control granulocytes as the effector cell, the titration was conducted using 0.5 µl of control whole blood. At a whole blood dilution of 1:4000, the effect of control complement introduced with the whole blood is negligible.

The results obtained using this simplified technique are encouraging, but the technique is presently undergoing further modification and testing.

At present, the laboratory data obtained in this set of experiments are being processed for comparison with the prospectively obtained clinical data. The final results of these studies will be submitted for publication.

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7. Allen RC: Direct Quantification of Phagocyte Oxygenation Activity in Whole Blood: A Chemiluminigenic Probe Aproach. J. Clin Chem Clin Biochem. 1981; 19: 583-583

PRESENTATIONS

Allen RC: Direct Quantification of Phagocyte Oxygenation Activity in Whole Blood: A Chemiluminigenic Probe Approach. (Invited Presentation Symposium 21 Luminescence). XI International Congress of Clinical Chemistry and IV European Congress of Clinical Chemistry, Vienna, Austria. Sep 1981

Allen RC: Chemiluminescence as a Tool for Defection of Dioxygenations. (Invited Presentation) International Conference on "Peroxides in Biological Systems" under the auspices of the European Society for Biochemical Pharmacology. Otzenhausen/Saar, Federal Republic of Germany, Sep 1981

Allen RC: The Information-Effector Relationship of Complement Activation to Stimulation of Phagocyte Oxygenation Activity as Measured by Chemiluminigenic Probing. 66th Annual Meeting of Federation of American Societies for Experimental Biology, New Orleans, LA, Apr 1982

Allen RC: Estimation of Phagocyte Oxygenation Activity by Chemiluminigenic Probing. 10th Annual Meeting American Society for Photobiology, Vancouver, British Columbia, Canada, Jun 1982

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Allen RC: Chemiluminescence and the Study of Phagocyte Redox Metabolism. In Biochemistry and Function of Phagocytes. F. Rossi and P. Patriarca (eds) Vol 141 Adv Exp Med Biol, Plenum Press, New York, 1982, pp 411-421

Allen RC and Pruitt BA Jr.: Humoral-Phagocyte Axis of Immune Defense in Burn Patients: Chemoluminigenic Probing. Arch Surg 117: 133-140, 1982

Allen RC: Biochemiexcitation: Chemiluminescence and the Study of Biological Oxygenation Reactions. In Chemical and Biological Generation of Excited States. W. Adam and G. Cilento (eds), Academic Press, New York, 1982, pp 309-344

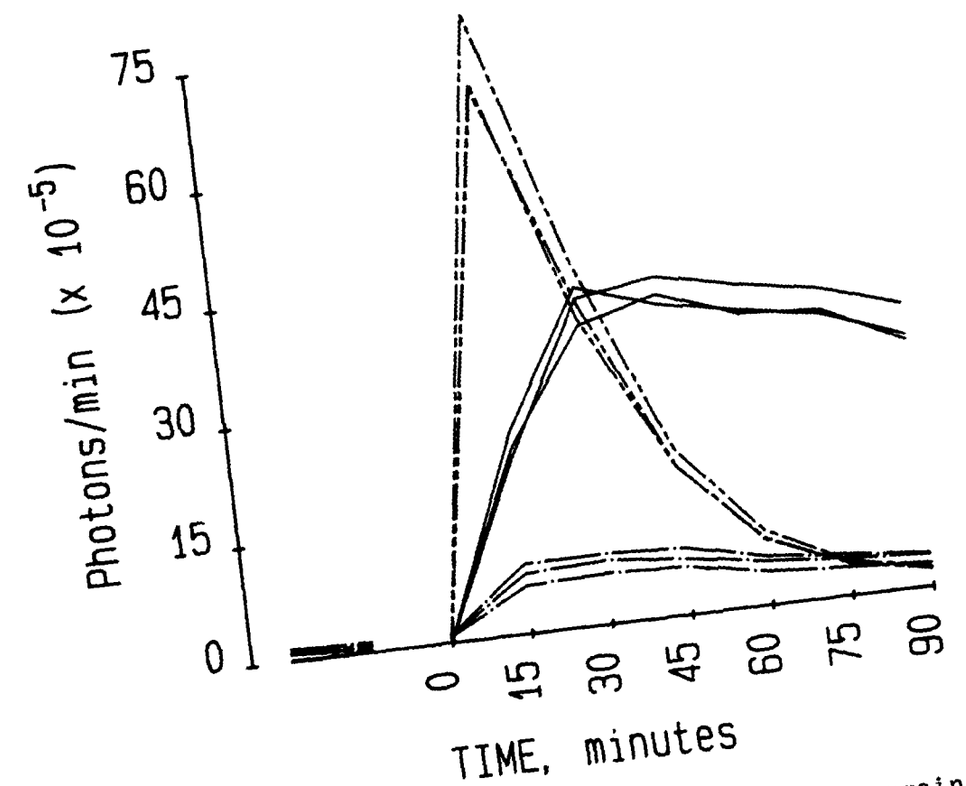

Figure 1. Chemiluminescent intensity measurements plotted against time. Triplicate measurements were taken for each condition of testing using 0.5 microliter of whole blood. The 0.5 µl blood specimen tested contained 1980 segmented PMNL, 670 band PMNL, 520 metamyelocytes, 160 myelocytes, 40 eosinophils, and 600 lymphocytes. The unbroken lines are the responses to opsonified zymosan using luminol (10 µM) as the CLP. The (——-- ——) and (——— · ———) lines are the response to phorbol myristate acetate using luminol (10 µM) and dimethyl biacridinium dinitrate (50 µM) as the CLP respectively. Time zero is the point of initiation of stimulation.

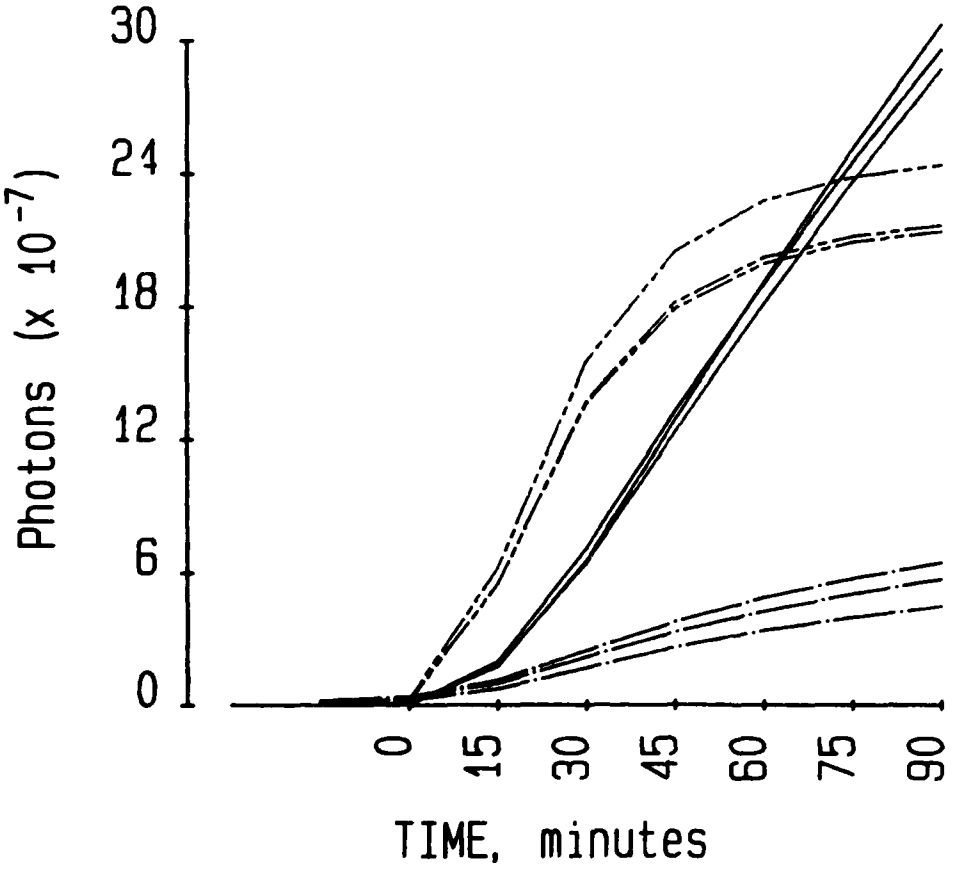

Figure 2. Integral chemiluminescent plotted versus time. The conditions are the same as described in Figure 1.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 3808	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8A. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	076
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Mechanisms of Opportunistic Infection in Burned Soldiers

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology and 012600 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 05	Cont	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	0.3	17
	CURRENT 83	0.3	21

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

ADDRESS: Fort Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL

NAME: Basil A. Pruitt, Jr., MD, COL, MC

TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

NAME: US Army Institute of Surgical Research Microbiology Branch

ADDRESS: Fort Sam Houston, Texas 78234

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: Albert T. McManus, Ph.D, MAJ, MSC

TELEPHONE: 512-221-3411

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

POC: DA

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Virulence Factors; (U) Plasmids; (U) Infection; (U) Toxins; (U) Antibiotics; (U) Pharmacologic Modulation; (U) Vaccines; (U) Lab Animal

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) Define mechanisms of microbial pathogenicity in burned soldier. Develop methods to combat specific virulence factors of opportunistic pathogens. Identify specific defects in immune defenses targeted by opportunistic pathogen. Develop methods to increase host resistance of burned soldiers to opportunistic infection.

24. (U) This project will examine both bacterial and host factors relating to opportunistic infection. A genetic approach will be used to investigate virulence mechanisms of bacteria taken from human burn infections. Isolates will be examined for the presence of extra chromosomal elements (plasmids) that might explain differences in strain virulence. Specific hypothesis about plasmids or chromosomal gene products as virulence factors will be investigated. Virulence mechanisms will be investigated in animal models. Knowledge of specific virulence mechanisms will be used to develop pharmacological, biological or physical means to disrupt micorbial virulence.

25. (U) 8110 - 8209. Plasmid DNA agarose electrophoresis patterns from 35 Providencia stuartii isolates have shown a consistent finding of a 34 Mdal plasmid. This plasmid is transferable to E. coli in vitro. A pilot study of the occurrence of transferable E. coli plasmids containing sulfonamide resistance genes has been initiated. This study has demonstrated that the appearance following admission of such plasmids is not an uncommon occurrence. Transferable resistance genes colinked with sulfonamide resistance include resistances to modern aminoglycosides, synthetic penicillins (including ureido-penicillins),

*Available to contractors upon originator's approval

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tetracylcines and chloramphenicol. A system for estimating bacterial host ranges and plasmid incompatability is being investigated. A computer aided identification of antibiotic resistance patterns associated with patient infections is being utilized to associate specific phenotypes with virulence.

AD-A133 132 US ARMY INSTITUTE OF SURGICAL RESEARCH ANNUAL RESEARCH PROGRESS REPORT FY 1982(U) ARMY INST OF SURGICAL RESEARCH FORT SAM HOUSTON TX B A PRUITT 01 OCT 82 4/5

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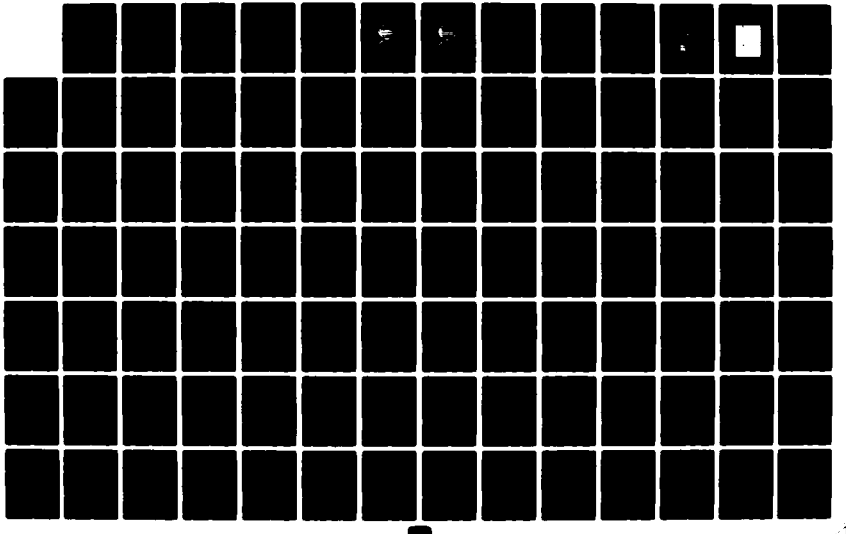

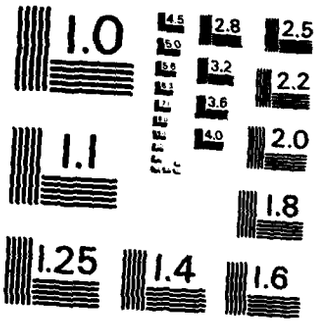

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00 IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: MECHANISMS OF OPPORTUNISTIC INFECTION IN BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1981 - 30 September 1982

Investigators:

Albert T. McManus, Ph.D., Major, MSC
Camille L. Denton, M.A.
Virginia C. English, M.A.
George T. Daye, Jr., M.A.
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00 IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: MECHANISMS OF OPPORTUNISTIC INFECTION IN BURNED SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1981 - 30 September 1982

Investigators: Albert T. McManus, Ph.D., Major, MSC
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Plasmid DNA agarose electrophoresis patterns from 35 Providencia stuartii isolates have shown a consistent finding of a 80 Mdal plasmid. This plasmid is transferable to Escherichia coli in vitro. A pilot study of the occurrence of transferable E. coli plasmids containing sulfonamide resistance genes has been initiated. This study has demonstrated that the appearance following admission of such plasmids is not an uncommon occurrence. Transferable resistance genes co-linked with sulfonamide resistance include resistances to modern aminoglycosides, synthetic penicillins (including ureido-penicillins), tetracyclines and chloramphenicol. A system for estimating bacterial host ranges and plasmid incompatability is being investigated. A computer aided identification of antibiotic resistance patterns associated with patient infections is being utilized to associate specific phenotypes with virulence.

Plasmids
Infection
Virulence factors
Antibiotics

MECHANISMS OF OPPORTUNISTIC INFECTION IN BURNED SOLDIERS

PROVIDENCIA STUARTII PLASMIDS

We reported on the isolation of an antibiotic resistance plasmid from an epidemic Providencia stuartii strain in the last reporting period (Annual Report FY 81, pp. 330-338). The plasmid has been further characterized and its molecular weight is now estimated at 80 million daltons. In addition, the genotype of the transferable aminoglycoside resistance mechanism has been identified as an AAC-(3)-11 enzyme (1). This enzyme is not a common mechanism of resistance in the United States. We are currently preparing a genetic probe specific for the gene producing this enzyme. This probe will facilitate the identification of this enzyme among aminoglycoside resistant burn patient flora. Such data will be helpful in establishing the epidemiology of the Providencia plasmid as well as identifying possible transposition or other genetic mechanisms of spread.

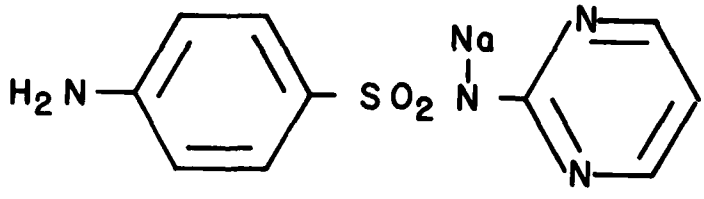
SULFONAMIDE RESISTANCE AMONG GRAM-NEGATIVE BURN FLORA

Sulfonamide resistance was greater than 78% in gram-negative isolates during this reporting period. This is a continuation of our previous experience. Sulfadiazine in silver-sulfadiazine is the only bacteriologically active sulfonamide used on our burn ward. We investigated the requirement of sulfadiazine for the in vitro activity of silver-sulfadiazine. A bacteriologically inactive analog of sulfadiazine was synthesized. The structure of sodium salts of sulfadiazine and the synthesized analog benzenesulfonamidopyrimidine (ISR-44) are presented in Figure 1. As can be seen, the two structures are very similar except that the analog does not have the N-4 amino group (para-amino) necessary for para-aminobenzoic acid antagonism. The silver salts of sulfadiazine and its analog were prepared. The two sodium salts and the two silver salts were then examined for in vitro and in vivo (burned rat) activity.

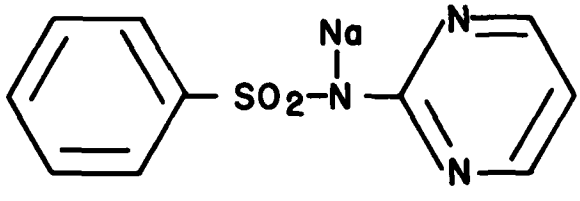
In vitro the compounds were examined against sulfonamide sensitive and sulfonamide resistant organisms. Compounds were examined in trench plates containing test compounds at 50 mg/ml of trench agar. Results with sulfonamide sensitive organisms are presented in Figure 2. The bottom two streaks are control organisms. As can be seen, sulfadiazine was active and analog (ISR-44) was not. On the other plate, however, the silver salts of both compounds showed activity against all strains. Results with sulfonamide resistant organisms are presented in Figure 3. Here, the sodium salts of both compounds (except for controls) were inactive. The silver salts, however, were active against all strains. From these data, silver appears to be the active component of silver-sulfadiazine in vitro.

Activities of the four test compounds were next examined in burned rats infected with an in vitro sulfadiazine sensitive virulent Pseudomonas

1. Miller GH, Sabatelli FJ, Hare RS, Waitz JA: Survey of aminoglycoside resistance patterns. Developments in Industrial Microbiology, Vol. 21, pp. 91-104, 1980.



Sulfadiazine Sodium



Benzenesulfonamidopyrimidine Sodium

Figure 1. Structural formulae of sulfadiazine sodium and its analog benzenesulfonamidopyrimidine sodium (ISR 44).

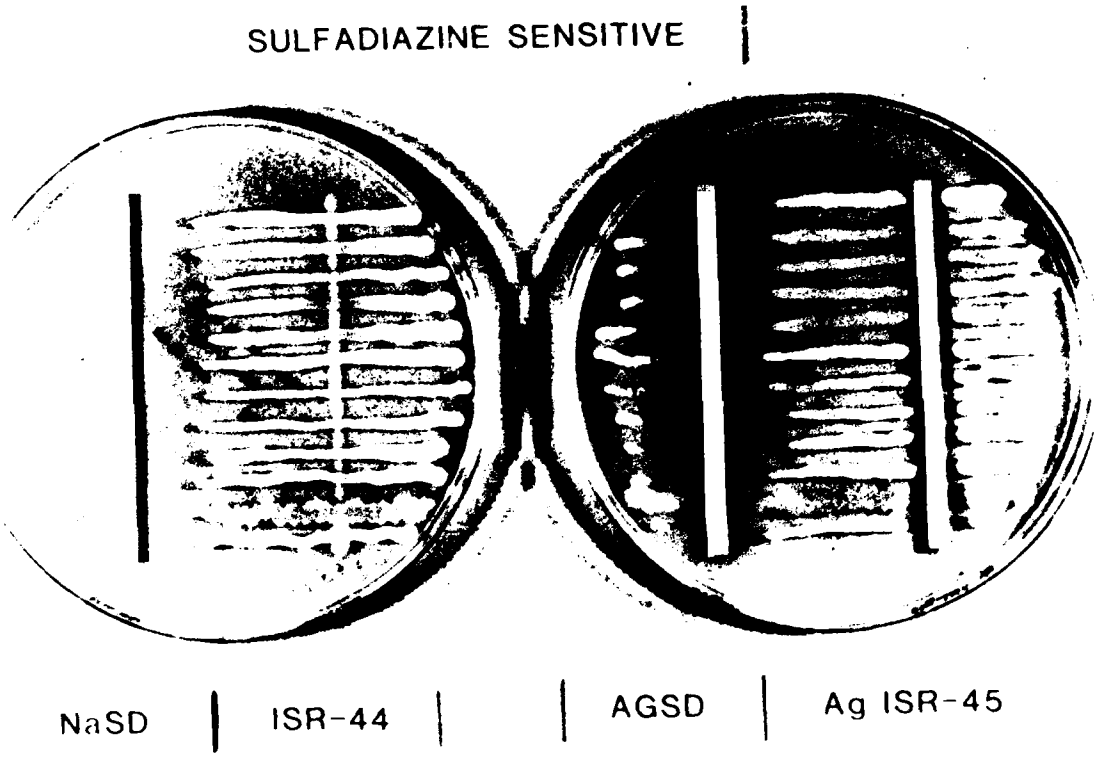


Figure 2. Trench plate assay for sensitivity of sulfonamide sensitive organisms to sulfadiazine sodium (NaSD) benzenesulfonamidopyrimidine sodium (ISR-44), sulfadiazine silver (AGSD), and benzenesulfonamido-pyrimidine silver (Ag ISR-45).

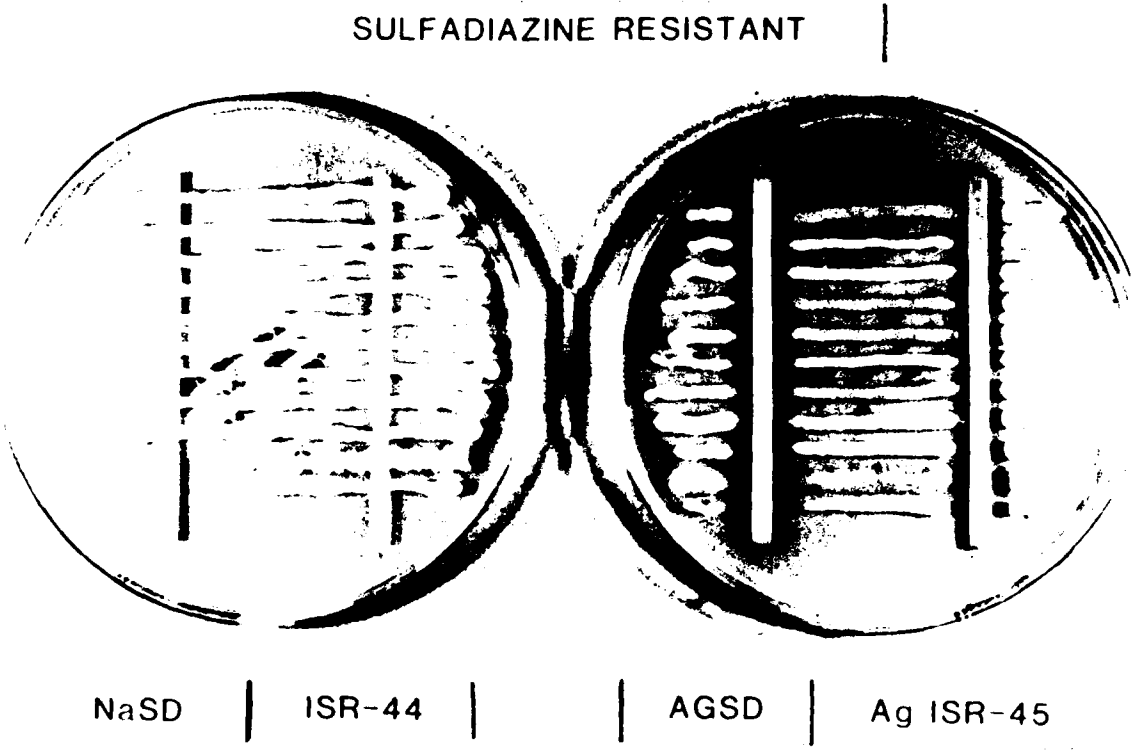


Figure 3. Trench plate assay for sensitivity of sulfonamide resistant organisms to sulfadiazine sodium (NaSD) benzenesulfonamidopyrimidine sodium (ISR-44), sulfadiazine silver (AGSD), and benzenesulfonamido-pyrimidine silver (Ag ISR-45).

aeruginosa (59-1244) and an in vitro sulfadiazine resistant virulent Pseudomonas aeruginosa (70-4189). The conformation of in vitro sensitivity of the strains is presented in Figure 4. For animal testing, animals (180 g male rats) were burned and inoculated using the standard Walker-Mason model. Chemotherapy was initiated 24 hours post burning and inoculation with 10^8 organisms. The in vivo effects of the compounds are presented in Table 1. These data show that sulfadiazine is an effective agent against invading sulfonamide sensitive Pseudomonas. The sulfonamide resistant invading strain was relatively resistant to sulfadiazine in vivo and was also resistant to the silver salts. These results indicate that silver is an ineffective agent against invading Pseudomonas aeruginosa. These data also speak to the limitations of interpretation of in vitro testing of silver-sulfadiazine.

SURVEY OF TRANFERABLE SULFONAMIDE RESISTANCE CONTAINING PLASMIDS IN PATIENT ESCHERICHIA COLI

A prospective study of the occurrence of transferable sulfonamide resistance plasmids in E. coli isolated during the hospital courses of 50 consecutively admitted burn patients was conducted. Patients were cultured twice weekly by rectal swab or from stool specimens. Escherichia coli was isolated on MacConkey's agar without added antibiotics. Isolated strains were then examined for sensitivity to sulfonamides. Strains found resistant were tested for transferable sulfonamide resistance by filter mating with a nalidixic acid resistant E. coli K12 strain C-600. Following mating and incubation, the cultures were selected for nalidixic acid resistant, sulfonamide resistant transconjugants. All patient isolates were sensitive to nalidixic acid. Transconjugant strains were tested for antibiotic resistance markers cotransferred without direct selection. Data are presented in Table 2. Of the 50 patients examined, all had E. coli recovered. Sulfonamide resistance occurred in 40 of the 50 patients. Of these 40 patient isolates, 22 strains contained sulfonamide resistance containing transferable plasmids.

A summary of antibiotic resistance mechanisms cotransferable with sulfonamide resistance genes is presented in Table 3. As can be seen, sulfonamide resistance containing plasmids are a serious risk.

In addition to antibiotic resistance patterns, the plasmids isolated above were examined for molecular weight profiles in agarose gel electrophoresis. An example of 11 strain patterns is presented in Figure 5. To date, no evidence for a single sulfonamide resistance containing plasmid has been found. The possibility of sulfonamide transposons is being investigated and will be reported.

ANTIBIOTIC RESISTANCE PATTERN SORTING

As mentioned in a separate section of this Annual Report, the clinical microbiology data are now stored in an automated data base. This base may be used to search for antibiotic sensitivity patterns as possible indicators of underlying plasmid spread. The antibiotic data are entered and stored

Table 1. Topical Chemotherapy in Experimental Pseudomonas Burn Infection of the Rat

Treatment[1]	Mortality (percent) Ps 59-1244	Ps 70-4189
Sulfadiazine sodium, 10 mg/g	0	90
Sulfadiazine sodium, 50 mg/g	0	50
ISR-44 (sodium), 50 mg/g	100	100
Sulfadiazine silver, 50 mg/g	0	80
ISR-45 (silver), 50 mg/g	100	100
Mafenide acetate, 112.5 mg/g	10	10
Infected (no other treatment)	100	100

[1] Treatment was initiated 24 hours after burning and inoculation and continued once per day for 10 days; 10 animals were used per group and mortality was recorded for 28 days.

Table 2. Occurrence of Sulfonamide Resistant E. coli in 50 Prospectively Studied Burn Patients[1]

	Number	Percent
Patients with E. coli isolated	50	100
Patients with demonstrable sulfonamide resistant E. coli	40	80
Patients with resistant E. coli that could sexually transfer sulfonamide resistance	22	44[2]

[1] Patients were followed from admission to acquisition of sulfonamide resistant E. coli. Strains were then examined for resistance transfer.

[2] Of resistant organisms, 55% had transferable plasmids.

Table 3. Antibiotic Resistance Markers Found at USAISR to be Associated with Transferable Sulfonamide Resistance Plasmids[1]

Aminoglycosides:	Gentamicin
	Tobramycin
	Netilmicin
	Neomycin
	Kanamycin
	Streptomycin
Penicillins:	Carbenicillin
	Ticarcillin
	Ampicillin
	Mezlocillin
	Piperacillin
Other classes:	Tetracycline
	Chloramphenicol
	Mercuric chloride

[1] Plasmid transferred to E. coli K-12 by filter mating and selection with sulfadiazine.

Table 4

SULFONAMIDE AND TICARCILLIN RESISTANT K.PNEUMO

PTN	DTAKEN	ANTIBIOTIC PATTERN	TIC1	MZ1	SD1
056 82	820606	SSSRSSSSSZSRZZZ	10.4	19.2	6.0
056 82	820608	SSSRSSSSSZSRZZZ	10.3	19.0	6.0
056 82	820609	SSSRSSSSSZSRZZZ	9.7	19.3	6.0
056 82	820612	SSSRSSSSSZSRZZZ	10.4	20.0	6.0
056 82	820613	SSSRSSSSSZSRZZZ	9.1	18.7	6.0
093 82	820606	SSSRSSSSSZSRZZZ	10.2	19.3	6.0
095 82	820606	SSSRSSSSSZSRZZZ	9.9	19.6	6.0
095 82	820612	SSSRSSSSSZSRZZZ	9.3	19.7	6.0
103 82	820626	SSSRSSSSSZSRZZZ	6.0	21.8	6.0
131 82	820806	SSSRSSSSSZSRZZZ	6.0	18.2	6.0
131 82	820814	SSSRSSSSSZSRZZZ	10.6	21.6	6.0
137 82	820815	SSSRSSSSSZSRZZZ	10.6	20.4	6.0
137 82	820821	SSSRSSSSSZSRZZZ	6.0	14.2	6.0
137 82	820821	SSSRSSSSSZSRZZZ	6.0	13.4	6.0
137 82	820902	SSSRSSSSSZSRZZZ	6.0	24.0	6.0
137 82	820903	SSSRSSSSSZSRZZZ	6.0	13.6	6.0
137 82	820911	SSSRSSSSSZSRZZZ	9.8	20.5	6.0
147 82	820901	SSSRSSSSSZSRZZZ	6.0	19.8	6.0
199 82	820728	SSSRSSSSSZSRZZZ	6.0	14.8	6.0
199 82	820728	SSSRSSSSSZSRZZZ	6.0	13.8	6.0
199 82	820729	SSSRSSSSSZSRZZZ	6.0	15.0	6.0

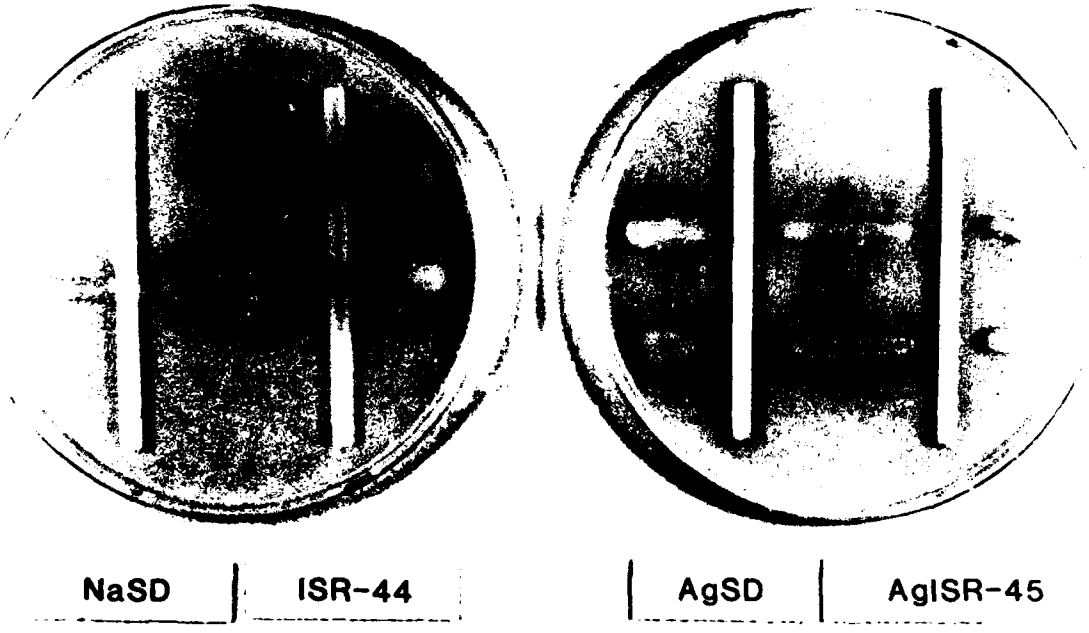


Figure 4. Trench plate assay for in vitro sensitivity of Pseudomonas aeruginosa strains 59-1244 (top streak) and 70-4189 (bottom streak).

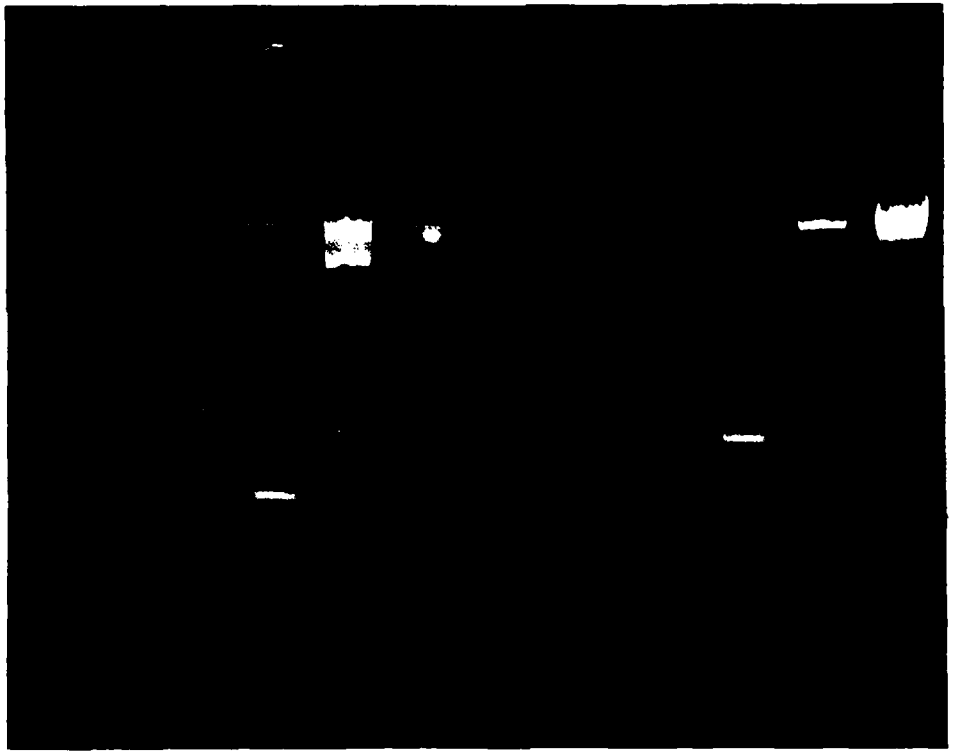


Figure 5. An example of agarose gel electrophoresis patterns of sulfonamide resistance plasmids transferred to E. coli C600.

as zone diameters (mm) of inhibition for each antibiotic. Each antibiotic sensitivity consists of 12 tests. All or any of the data can be used to search for patterns. An example of a search for Klebsiella pneumoniae with the resistance pattern of sulfonamide resistance and ticarcillin resistance is presented in Table 4. The data are presented by patient number (PTN), date of culture (DTAKEN) and antibiotic pattern as sensitive (S) or resistant (R), or not tested = zero (Z) for the string of antibiotics (Position 4 = ticarcillin, Position 5 = mezlocillin, Position 12 = sulfonamide). The table also contains the inhibition zones for ticarcillin (TIC1), mezlocillin (MZ1) and sulfadiazine (SD1) which were used to delineate the organisms as resistant or sensitive. Note that the ticarcillin resistant phenotype was not cross resistant to the newer semisynthetic penicillin mezlocillin. The utility of this sorting technique is still being explored and will be reported in future reports.

SEROLOGIC TYPES OF PSEUDOMONAS AERUGINOSA FOUND IN BURN PATIENTS

A total of 385 strains, collected from 66 patients, were typed using the International Typing Set (DIFCO). Strains were selected by the antibiotic pattern sorting technique noted above. Each pattern type per patient per month was serotyped. The distribution of serotypes is presented in Table 5. As can be seen, Type 15 was the major serotype present. This is a continuation of findings of the past six reporting periods. Type 15 represents the endemic flora of Pseudomonas aeruginosa.

PRESENTATIONS

McManus AT: Studies on the mechanisms of in vitro sensitivity to sulfadiazine silver. Annual Meeting of the Surgical Infection Society, Boston, Massachusetts, 19 April 1982.

PUBLICATIONS

McManus AT, Denton CL, Mason AD, Jr: Mechanisms of in vitro sensitivity to sulfadiazine silver. Arch Surg, in press.

Table 5. Serotypes of Strains of *Pseudomonas aeruginosa* from 66 Burn Patients in 1982

Type	No. of Strains
1	8
3	1
5	2
6	52
6,9	2
6,10	1
6,15	4
7	8
7,11	1
9,10	2
10	7
10,15	1
11	11
11,15	1
12	3
12,6	1
15	254
15,6	5
15,10	1
Non-typable	20
Total	385

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOG 9551	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 01 29	H.TERM	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91CLA	00	081
b. CONTRIBUTING				
c. [illegible]	None			

11. TITLE (Procede with Security Classification Code)

(U) Characterization of Biochemical Indicators of Infection in the Thermally Injured

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry and 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 10	82 06	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	0.6	12
FISCAL YEAR CURRENT 83	0.0	0

19. RESPONSIBLE DOD ORGANIZATION

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RESPONSIBLE INDIVIDUAL

NAME: Basil A. Pruitt, Jr., COL, MC

TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)

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TELEPHONE: 512-221-4106

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME: POC: DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Thermal Injury; (U) Infection; (U) Indicators of Infection; (U) Plasma; (U) Erythrocytes; (U) Proteins

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) To characterize, purify and identify the biochemical indicators of infection found in perchloric acid filtrates of whole blood taken from burned-infected individuals. To elucidate the interactions between plasma from burned-infected animals and erythrocytes which give rise to two of the biochemical indicators of infection.

24. (U) In order to characterize, purify and identify the biochemical indicators of infection, a number of approaches/methods will be used: (a) Physical and chemical techniques generally employed to purify and characterize proteins. (b) Incubation of plasma or whole blood containing the biochemical indicators with various enzymes and reagents, which are likely to affect one or more of the components of the indicators and/or the generation of the indicators. (c) Incubation of plasma from burned-infected animals with components of erythrocytes and chemical analogs to establish which components of erythrocytes interact with plasma factors to generate the 398 nm absorbance and 355/420 fluorescence factors.

25. (U) 8110 - 8206 . Heme-containing compounds appear to be able to substitute for erythrocytes in the generation of the OD 398 and fluorescence 355/420 biochemical indicators. This may allow us to retrospectively analyze patient plasma. Heat treatment, ammonium sulfate fractionation and Sephadex column chromatography have been used

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

to further characterize and purify the plasma protein factors which interact with erythrocytes to produce the OD 398 and fluorescence 355/420 indicators. It appears that the plasma protein component has a molecular weight of 70,000 daltons and may exist in normal plasma in a trimeric form. Though there is a slight effect of age on the biochemical indicators of infection, there is little change if one is studying mature animals. D-galactosamine induced liver damage suppresses the OD 398 and fluorescence 280/340 responses suggesting that these factors may be of hepatic origin. The 355/420 factor is not affected. $HgCl_2$ induced renal failure produces slight increases in OD 398 and fluorescence 280/340 but a 5 fold increase in fluorescence 355/420. The increase in 355/420 appears to be due to a plasma borne factor not involved in the generation of the biochemical indicators of infection. Elevated BUN levels have no effect on the biochemical indicators.

TERMINATION REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: CHARACTERIZATION OF BIOCHEMICAL INDICATORS OF INFECTION IN THE THERMALLY INJURED

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1981 - 15 July 1982

Investigators:

Michael C. Powanda, Ph.D., Lieutenant Colonel, MSC
John Dubois, B.S.
Ysidro Villarreal, B.S.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00 IN-HOUSE INDEPENDENT LABORATORY RESEARCH

REPORT TITLE: CHARACTERIZATION OF BIOCHEMICAL INDICATORS OF INFECTION IN THE THERMALLY INJURED

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1981 - 15 July 1982

Investigators: Michael C. Powanda, Ph.D., Lieutenant Colonel, MSC
John Dubois, B.S.
Ysidro Villarreal, B.S.

Reports Control Symbol MEDDH-288(R1)

Heme-containing compounds appear to be able to substitute for erythrocytes in the generation of the OD 398 and fluorescence 355/420 biochemical indicators. This may allow us to analyze stored patient plasma. Heat treatment, ammonium sulfate fractionation and Sephadex column chromatography have been used to characterize and purify the plasma protein factors which interact with erythrocytes to produce the OD 398 and fluorescence 355/420 indicators. It appears that the plasma-protein component has a molecular weight of 70,000 daltons and may exist in normal plasma in a trimeric form. Though there is a slight effect of age on the biochemical indicators of infection, there is little variance within mature animals. D-galactosamine-induced liver damage suppresses the OD 398 and fluorescence 280/340 responses, suggesting that these factors may be of hepatic origin. The 355/420 factor is not affected. $HgCl_2$-induced renal failure produces slight increases in OD 398 and fluorescence 280/340 but a fivefold increase in fluorescence 355/420. The increase in 355/420 appears to be due to a plasma-borne factor not involved in the generation of the biochemical indicators of infection. Elevated BUN levels have no effect on the biochemical indicators.

Analysis of plasma samples from well characterized patients for selected plasma proteins reveals that there are significant differences in some of these proteins between burned and burned-infected patients with or without complications. Unfortunately the ranges of the values for these proteins overlap somewhat, thus diminishing their value as indicators of infection in severely burned patients. However, the ratios of α_1-acid glycoprotein/haptoglobin, α_1-acid glycoprotein/transferrin, and to a lesser degree α_1-acid glycoprotein/Igm and α_1-acid glycoprotein/α_2-macroglobulin do appear to be very effective discriminators between injured and injured-infected patients.

Thermal injury
Infection
Erythrocytes
Indicators of infection
Plasma
Proteins

CHARACTERIZATION OF BIOCHEMICAL INDICATORS OF INFECTION IN THE THERMALLY INJURED

INTRODUCTION

The treatment of severe thermal trauma is very often complicated by infection which occurs readily in such patients (1,2). The loss of the skin barrier and the extensive metabolic and physiologic alterations in burn patients render the detection of infection more difficult and may allow wound colonization to be mistaken for systemic infection. In the course of assaying perchloric acid filtrates of whole blood for various metabolites to determine if a metabolic profile could be established which would discriminate burned-infected rats from burned-noninfected rats, three factors were found, two of which appear to be useful indicators of the presence of infection (3,4). The following presents the results of attempts to purify and identify these two factors and the conditions of their generation, as well as assessing the effects of age, liver damage and acute renal failure on the amounts of these factors in circulating blood.

Marked alterations in the concentration of selected plasma proteins occur following injury and during infection (5,6). There is evidence to suggest that a number of the plasma proteins may be involved in wound healing (5) and host defense against infection (6). It is conceivable that the pattern of the alterations in the concentrations of some of the plasma proteins might differ in injured and injured-infected patients. The following also presents data indicating that the ratios of selected plasma proteins may distinguish between burned and burned-infected patients.

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1. McManus WF, Goodwin CW, Mason AD Jr, Pruitt BA Jr: Burn wound infection. J Trauma 21:753-756, 1981.

2. McManus WF, et al: Clinical Operation, Center for Treatment of Burned Soldiers. US Army Institute of Surgical Research Annual Research Progress Report, FY 1980, pp. 1-28.

3. Powanda MC, Dubois J, Villarreal Y, Walker HL, Pruitt BA Jr: Detection of potential biochemical indicators of infection in the burned rat. J Lab Clin Med 97:672-679, 1981.

4. Powanda MC, Dubois J, Villarreal Y, Lieberman MM, Pruitt BA Jr: Biochemical indicators of infection and inflammation in burn injury. In Proceedings, 13th Army Science Conference, held at the US Military Academy, West Point, New York, June 1982.

5. Powanda MC, Moyer ED: Plasma proteins and wound healing. Surg Gynecol Obstet 153:749-755, 1981.

6. Powanda MC, Moyer ED: Plasma protein alterations during infection: Potential significance of these changes to host defense and repair systems. In Infection: The Physiologic and Metabolic Responses of the Host. M.C. Powanda and P.G. Canonico (eds.), Elsevier/North Holland Publishing Company, Amsterdam, 1981, pp. 271-296.

METHODS AND MATERIALS

The rats used in attempts to purify and identify the biochemical indicators of infection and for the liver damage and acute renal failure studies were obtained either from Holtzman or from Timco. The standard burn model of Walker and Mason (7) was used to generate 30% full-thickness scald injuries on the dorsal surface. Pseudomonas aeruginosa strain 12-4-4 was used to infect the burned rats. The biochemical indicators were quantitated as previously described (3) except that 0.2 ml of 30% H_2O_2 was added prior to the measurement of the 355/420 fluorescent factor. Details of the purification procedures used are given below.

The rats used to determine the effect of age on the biochemical indicators of infection were from the Southwest Foundation for Research and Education A X C colony.

Plasma protein analyses were done using the Hyland laser nephelometer and specific antibodies bought from Hyland.

RESULTS AND DISCUSSION

The discovery that erythrocytes were one of the components responsible for the generation of the 398 and 355/420 indicators of infection (4,8) led us to test whether hemoglobin or other heme-containing substances could participate in the generation of these indicators. Table 1 demonstrates that hemoglobin, methemoglobin, myoglobin and even hemin can all interact with plasma from burned-infected animals to generate the 398 nm indicator. Except for methemoglobin, all of these compounds can participate equally well in the production of the 355/420 fluorescent indicator. The inability of methemoglobin to generate appreciable quantities of 355/420 material does not seem to be due to the presence of iron in the ferric form since this is also true of hemin. None of the compounds, except for hemoglobin, generates much of the 398 nm or 355/420 indicators when mixed with saline instead of plasma and even hemoglobin only produces some 355/420 fluorescence but little or no 398 absorbance. Though it appears that heme-containing compounds could substitute for erythrocytes in the assay of plasma for the 398 nm and the 355/420 indicators, it seemed advisable to continue to use erythrocytes until the plasma components had been purified and identified to eliminate the possibility of spurious results.

The findings that the source of plasma was the critical factor in the generation of the 398 and 355/420 indicators and that erythrocytes appear to be the cells which interact with the plasma substances in the presence of PCA to produce these indicators (4,8) allowed us to pursue the

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7. Walker HL, Mason AD Jr: A standard animal burn. J Trauma 8:1049-1051, 1968.

8. Powanda MC, Dubois J, Villarreal Y: Monitoring and Modification of the Metabolic and Physiologic Alterations Associated with Thermal Injury in Burned Soldiers. US Army Institute of Surgical Research Annual Research Progress Report, FY 1981, pp. 339-352.

following approach to the characterization and identification of the indicators. Rather than having to work with an unstable acid filtrate of whole blood, we could use plasma from burned-infected animals and employ classical techniques for the purification of proteins. The samples resulting from these procedures could then be assayed for the presence of the indicators by adding erythrocytes from normal animals, followed by PCA. We first tried selective heat denaturation followed by ammonium sulfate fractionation. We found we could heat the plasma at 60° C for 30 minutes with no loss of activity (Table 2), but with about a 30% decrease in total protein content. The fact that the indicators were resistant to this treatment indicated that complement, which is inactivated under these conditions, is unlikely to be involved in the generation of the indicators. One hundred milliliters of pooled plasma from rats 4 days postinjury plus infection were heat treated and then subjected to $(NH_4)_2SO_4$ fractionation at room temperature (22-24° C). The resulting precipitates were solubilized in 40 ml of 0.9 N saline and assayed using RBCs from normal rats. The preponderance of 398 and 355/420 generating factors could be found in the 40-60% saturation range, with some tailing into the 60-80% fraction (Table 2). In contrast, fluorescence 280/340 was polydisperse, with the greatest amount being found in the 60-80% fraction but with considerable such fluorescence detectable in the 20-40% and 40-60% fractions. The polydisperse nature of the 280/340 fluorescence is consistent with such fluorescence resulting from the presence of tryptophan in the protein and the fact that most proteins contain tryptophan. Thus it would appear that changes in 280/340 which occur in response to injury as well as infection reflect changes in the concentration of more than a single protein.

Since the 40-60% $(NH_4)_2SO_4$ fraction did not contain all of the 398 and 355/420 plasma protein factors, another 100 ml aliquot of plasma from burned-infected rats was heat treated and $(NH_4)_2SO_4$ added to produce a 35-70% fraction. This fraction was redissolved in 30 ml of saline and 15 ml of the solution were applied to a 90 x 4.4 cm calibrated Sephacryl S-200 column. Normal saline was used to elute, and 10 ml fractions were collected. Plasma from unburned, uninfected rats was similarly treated. The data from both column separations are presented as a composite in Figure 1. Not surprisingly, plasma from burned-infected rats displays a somewhat different molecular weight distribution of proteins than does that from normal animals (top left). Consistent with this altered protein distribution is the shift in fluorescence 280/340 towards lower molecular weight forms in the plasma from burned-infected rats (bottom left). Totally unexpected was the fact that plasma from control animals contained a protein (or proteins) which reacted with RBCs to generate the 398 and 355/420 indicators (right). The protein component(s) of these indicators detectable in normal plasma has (have) a molecular weight of approximately 210,000-230,000 daltons in contrast to that found in burned-infected plasma which appears to have a MW of approximately 70,000 daltons. It is conceivable that there is a polymeric form of the protein component of these indicators of infection present in normal plasma which is not detectable when perchloric acid is added to whole blood, but which is converted to a monomeric assayable form during infection. Preliminary data, not shown, hint that such a conversion could be accomplished by proteases,

Table 1. The Potential Role of Heme-containing Compounds in the Generation of Two of the Biochemical Indicators of Infection

Source of Ligand	+ Plasma		+ Saline	
	OD 398	355/420	OD 398	355/420
Cells	.745 ± .021	169 ± 1	.062 ± .004	7.3 ± 0.5
Hemoglobin	.580 ± .013	119 ± 4	.066 ± .010	36.0 ± 3.0
Methemoglobin	.495 ± .009	32 ± 1	.093 ± .003	6.3 ± 0.9
Myoglobin	.330 ± .011	145 ± 2	.043 ± .003	6.0 ± 0.4
Hemin	.819 ± .014	140 ± 2	.056 ± .005	1.8 ± 0.3

n = 4; mean ± SEM; additions of ligands (other than cells) were 0.5 ml of a 2.3 mM solution

Table 2. $(NH_4)_2SO_4$ Fractionation of Biochemical Indicators of Infection

Sample	OD 398 nm	Fluorescence 280/340	Fluorescence 355/420
Untreated plasma	.157	3700	80
	.167	3700	81
60°, 30' plasma	.157	3650	82
	.160	3700	84
0-20% $(NH_4)_2SO_4$	.024	320	9
	.027	350	11
	.025	300	8
20-40% $(NH_4)_2SO_4$	.110	2450	36
	.118	2500	38
	.119	2475	38
40-60% $(NH_4)_2SO_4$	.529	2700	355
	.512	2600	360
	.500	2650	350
60-80% $(NH_4)_2SO_4$	.223	4950	73
	.204	5000	71
	.199	5000	73
Remainder	.021	250	9
	.023	270	8
	.022	280	10

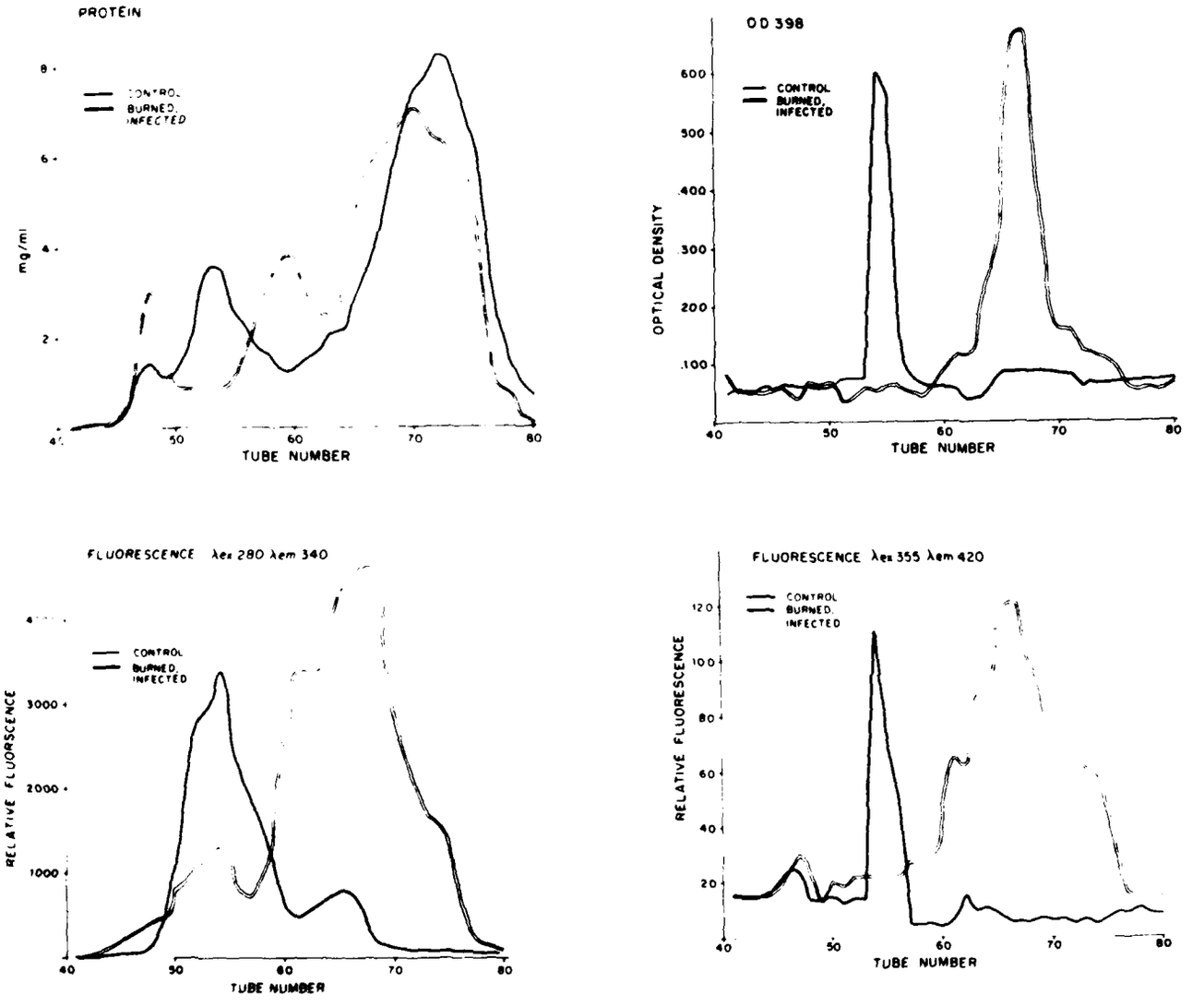
PROTEIN
CONTROL
BURNED,
INFECTED
mg/ml
TUBE NUMBER
OD 398
OPTICAL DENSITY
FLUORESCENCE λex 280 λem 340
RELATIVE FLUORSCENCE
FLUORESCENCE λex 355 λem 420
RELATIVE FLUORESCENCE

Figure 1

though chemicals such as urea, which induce unfolding of proteins, can also enhance the detection of the 398 and 355/420 indicators. Preliminary sodium dodecyl sulfate acrylamide gel electrophoresis suggests that there is a protein present in the Sephacryl S-200 column fractions of burned-infected plasma which is not detectable in these fractions when normal plasma is passed through the column. The estimated molecular weight of this protein is 46,000-47,000 daltons. It remains to be determined whether this protein is the, or one of the, component(s) of the 398 and 355/420 biochemical indicators of infection. The difference in the apparent molecular weight may be due to mode of separation.

In the course of the numerous studies on the biochemical indicators of infection, both young (approximately 45 days of age) and mature (90 days of age or more) rats were used and there appeared to be slight but real differences in the control animals' values for the 398 and 355/420 factors. To determine if age does affect these variables and to eliminate the possibility that the slight variations we observed from study to study were not due to techniques or to animal transport care, or housing, a study was carried out with the assistance of Dr. Sidney A. Shain of the Southwest Foundation for Research and Education. The SWFRE A X C colony is a well-characterized rat colony used for aging studies and allowed us to simultaneously sample rats ranging in age from 1 to 24 months. Table 3 shows that young healthy rats (30 days of age) do indeed have significantly lower 398 values and higher 355/420 values than older ($\geq$ 90-day) rats. Otherwise there are no significant effects of age upon these two variables. Thus as long as one is studying mature animals there need be little concern about the effect of age on these variables. The differences between 30 and $\geq$ 90 day rats may be due to a difference in the plasma protein component or to the red cell component of these indicators. These data also provide additional circumstantial evidence that the 398 factor and the 355/420 factor are not the same, since they respond to age in an opposite manner. Fluorescence 280/340 appears to exhibit a biphasic response, peaking at 3 months and tapering off after 12 months of age.

Since many of the metabolic indices of infection are likely to be affected by other forms of inflammation (9), a non-infectious hepatitis model (10) was employed to assess whether the putative biochemical indicators of infection would respond to liver damage. Animals were injected intraperitoneally with D-galactosamine and bled out at 18, 42 and 66 hours postinjection of the hepatotoxin. Only the 42-hour data are shown (Table 4), since this was the time of maximal increase in serum glutamic oxaloacetic transaminase which reflects the extent of tissue damage. As can be seen, both OD 398 and fluorescence 280/340 are reduced, rather than increased, by D-galactosamine treatment. There appears to be an inverse

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9. Powanda MC: The role of leukocyte endogenous mediator (endogenous pyrogen) in inflammation. In Inflammatory Diseases and Copper. John R.J. Sorenson (ed.), The Humana Press, Clifton, New Jersey, 1982, pp. 31-43.

10. Müller-Berghaus G, Reuter C, Bleyl U: Experimental galactosamine-induced hepatitis: Effect of anticoagulant and antifibrinolytic agents on microclot formation. Am J Pathol 82:393-406, 1976.

Table 3. Effect of Age on the Biochemical Indicators of Infection

Indicator	Age in Months					
	1	3	6	12	18	24
Optical density 398 nm	.081 ±.004	.128[a] ±.007	.144[a] ±.006	.130[a] ±.005	.148[a] ±.005	.152[a] ±.003
Fluorescence λ280/λ340	231 ± 5	265 ± 7	241 ± 8	216[b] ± 6	202[b] ± 8	196[b] ± 9
Fluorescence λ355/λ420	37.3 ± 1.7	33.4[a] ± 2.5	25.7[a] ± 0.4	30.0[a] ± 0.5	29.0[a] ± 0.9	29.5[a] ± 0.6

Mean ± SEM; n = 8 except for F 280/340, F 355/420 at 6 months, n = 7.
Analysis of variance was used to determine significance.
[a] $P \leq 0.01$ vs 1-month values.
[b] $P \leq 0.01$ vs 3-month values.

Table 4. Effect of D-galactosamine-induced Liver Damage on the Production of the Biochemical Indicators of Infection

Group	SGOT IU/L	OD 398	Fluorescence 280/340	Fluorescence 355/420
Control	55 ± 6	.091 ±.007	330 ±10	16 ±1
200 mg/kg D-gal-NH_2	125 ± 27	.066 ±.004	263 ±11	15 ±1
400 mg/kg D-gal-NH_2	1845 ±719	.043 ±.004	220 ± 9	14 ±1

Mean ± SEM; n = 6; SGOT = serum glutamic oxaloacetic transaminase in international units/liter; D-gal-NH_2 injected ip 42 hours previously.

relationship between the log of SGOT activity and OD 398 and fluorescence 280/340, suggesting that these two factors may be of hepatic origin and their product and/or release is inhibited by hepatic dysfunction. There is little or no change in fluorescence 355/420 induced by D-galactosamine which may indicate that this factor is not of hepatic origin and is different from the 398 and 280/340 factors.

The effect of renal damage induced by intramuscular injections of $HgCl_2$ (11) on the biochemical indicators is shown in Table 5. Forty-eight hours after the injection of $HgCl_2$, the BUN and creatinine levels are increased tenfold. There is a 50% increase in OD 398 and fluorescence 280/340 and a fivefold increase in fluorescence 355/420. However, much of the increase in fluorescence 355/420 appears to be due to a plasma-borne factor and not the result of the interaction of erythrocytes and plasma as is the case for the biochemical indicators. The plasma-borne 355/420 factor may be the same one detected in patients with chronic renal disease (12). Addition of urea to whole blood sufficient to produce a BUN of 300 does not produce any of the changes seen in induced renal failure.

Though the following data were generated under the work unit "Monitoring and Modification of the Metabolic and Physiologic Alterations Associated with Thermal Injury in Burned Soldiers," the data analysis which leads us to believe that the ratios of selected plasma proteins may be of use in helping clinicians discriminate between injured and injured-infected patients has only been completed recently.

As part of a study of liver metabolism in burned (B) and burned-infected patients, without or with complications (BI, BIC) conducted by Dr. Wilmore et al (13), trans-hepatic measurements of selected plasma proteins were made to assess the contribution of these proteins to nitrogen turnover in these patients. In addition to the arteriovenous sampling across the liver, peripheral vein blood samples were taken for plasma protein determinations. A detailed analysis of these peripheral vein protein data from these exceedingly well-characterized, age and burn size matched patients (13) has allowed us to ask whether the plasma concentration of any of these proteins would aid in distinguishing the burned-infected from the burned-noninfected patient. The concentrations of α_1-acid glycoprotein, haptoglobin and Igm are significantly different in burned versus burned-infected patients without complications (Table 6). The concentration of

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11. de Rougemont D, Wunderlich PF, Torhorst J, Keller M, Peters-Häfeli L, Thiel G, Brunner FP: $HgCl_2$-induced acute renal failure in the rat: Effects of water diuresis, saline loading, and diuretic drugs. J Lab Clin Med 99:646-656, 1982.

12. Schwertner HA, Hawthorne SB: Albumin-bound fluorescence in serum of patients with chronic renal failure. Clin Chem 26:649-652, 1980.

13. Wilmore DW, Goodwin CW, Aulick LH, Powanda MC, Mason AD Jr, Pruitt BA Jr: Effect of injury and infection on visceral metabolism and circulation. Ann Surg 192:491-504, 1980.

Table 5. Effect of Induced Renal Damage on Biochemical Indicators of Infection

Group	BUN mg/dl	Creatinine mg/dl	OD 398	Fluorescence 280/340 Plasma	Fluorescence 355/420 Blood	Fluorescence 355/420 Plasma
Saline (6)	22 ± 1	0.58 ±0.02	0.141 ±0.003	798 ± 28	28 ± 1	12 ±1
2.5 mg $HgCl_2$ per 100 g body wgt im (8)	216 ±16	5.87 ±0.18	0.207 ±0.008	1194 ± 42	141 ± 6	95 ±5

Mean ± SEM.

Table 6. Patient Groups

Protein (mg/dl)	Burned (B) [6]	Burned-infected (BI) [5]	Burned-infected with complications (BIC) [5]
α_1-antitrypsin	629 ± 42 (447 - 727)	452 ± 79 (307 - 749)	692 ± 108 (517 - 1023)
α_1-acid glycoprotein	105 ± 20[b,c] (33 - 155)	282 ± 13 (234 - 306)	235 ± 37 (153 - 352)
C-reactive protein	26.8 ± 3.5[c] (17.8 - 39.8)	24.9 ± 3.7 (17.8 - 38.0)	14.1 ± 1.8 (7.5 - 18.0)
Haptoglobin	371 ± 41[b] (216 - 461)	144 ± 8 (115 - 160)	214 ± 44 (99 - 355)
Transferrin	176 ± 24 (98 - 238)	92 ± 5 (76 - 102)	117 ± 30 (59 - 234)
α_2-macroglobulin	136 ± 25 (72 - 212)	95 ± 24 (55 - 184)	76 ± 13 (46 - 113)
Igm	164 ± 40[a] (98 - 359)	33 ± 9 (12 - 63)	71 ± 28 (9 - 175)

Mean ± SEM; range indicated in parentheses; [ ] = n of pts.
Data were rank ordered; analysis of variance was used to determine significance.
Comparisons: B vs BI, a = $P < 0.01$, b = $P < 0.001$.
B vs BIC, c = $P < 0.01$, d = $P < 0.001$.
BI vs BIC, e = $P < 0.01$; f = $P < 0.001$.

α_1-acid glycoprotein in burned patients is also significantly lower than in burned-infected patients with complications, while the concentration of C-reactive protein in burned patients, though not significantly different from that in infected patients without complications, is greater than that found in infected patients with complications. Even though there are some highly significant differences in the concentrations of certain plasma proteins between groups of infected and uninfected burned patients, it would not always be possible to use the concentration of these proteins to determine if a given burned individual were infected or not, since there is some degree of overlap in the range of values for these proteins, especially for the burned and burned-infected patients with complications. However, knowing the concentration of α_1-acid glycoprotein and that of haptoglobin, transferrin, IGM or α_2-macroglobulin in a burned patient's plasma does allow one to calculate a ratio which appears to distinguish clearly which patients are infected from those who are not (Table 7). There is no overlap amongst any of the ratios, and so all of them appear to be effective discriminators of the presence of infection. However, as the range and standard error values indicate, the α_1-acid glycoprotein/haptoglobin and the α_1-acid glycoprotein/transferrin ratios are likely to be the most effective delineators.

PUBLICATIONS

Powanda MC, Moyer ED: Plasma protein alterations during infection: Potential significance of these changes to host defense and repair systems. In Infection: The Physiologic and Metabolic Responses of the Host. M.C. Powanda and P.G. Canonico (eds.), Elsevier/North Holland Publishing Company, Amsterdam, 1981, pp. 271-296.

Powanda MC, Beisel WR: Hypothesis - Leukocyte endogenous mediator/endogenous pyrogen/lymphocyte activating factor modulates the development of nonspecific and specific immunity and affects nutritional status. Am J Clin Nutr 35:762-768, 1982.

Powanda MC: The role of leukocyte endogenous mediator (endogenous pyrogen) in inflammation. In Inflammatory Diseases and Copper. John R.J. Sorenson (ed.), The Humana Press, Clifton, New Jersey, 1982, pp. 31-43.

Powanda MC, Dubois J, Villarreal Y, Lieberman MM, Pruitt BA Jr: Biochemical indicators of infection and inflammation in burn injury. In Proceedings, 13th Army Science Conference, held at the US Military Academy, West Point, New York, June 1982.

Powanda MC, Moyer ED: Selected aspects of protein metabolism in relation to reticuloendothelial system, lymphocyte and fibroblast function. In The Reticuloendothelial System: A Comprehensive Treatise, Vol. 4 - Physiology of the Reticuloendothelial System. S.M. Reichard and J.P. Filkins (eds.), Plenum Press, New York. In press.

PRESENTATIONS

Powanda MC: The potential value of selected plasma proteins in host resistance to infection and wound healing. Surgical Grand Rounds, Erie County Medical Center, Buffalo, New York, 20 March 1982.

Powanda MC: The role of leukocyte endogenous mediator/endogenous pyrogen/lymphocyte activity factor in the host response to injury and infection. Trauma/Metabolism Research Group, Erie County Medical Center, Buffalo, New York, 22 March 1982.

Powanda MC: The role of leukocyte endogenous mediator/endogenous pyrogen/lymphocyte activating factor in nonspecific and specific immunity. Department of Medical Microbiology and Immunology, Texas A&M University, College Station, Texas, 25 March 1982.

Powanda MC: Partial characterization of some biochemical indicators of infection. Sixty-sixth Annual Meeting, Federation of American Societies for Experimental Biology, New Orleans, Louisiana, 19 April 1982.

Table 7. Patient Groups

Ratios of Proteins	Burned (B) [6]	Burned-infected (BI) [5]	Burned-infected with complications (BIC) [5]
α_1-acid glycoprotein/haptoglobin	0.273 ± 0.037[b,d] (0.153 - 0.392)	1.983 ± 0.176[e] (1.708 - 2.661)	1.165 ± 0.123 (0.923 - 1.545)
α_1-acid glycoprotein/transferrin	0.572 ± 0.052[b,d] (0.337 - 0.697)	3.105 ± 0.232[e] (2.819 - 4.026)	2.188 ± 0.266 (1.504 - 2.772)
α_1-acid glycoprotein/Igm	0.766 ± 0.204[b,c] (0.231 - 1.582)	12.516 ± 3.945 (4.857 - 25.083)	6.477 ± 2.785 (2.011 - 17.000)
α_1-acid glycoprotein/α_2-macroglobulin	0.775 ± 0.086[b,d] (0.440 - 1.046)	3.609 ± 0.718 (1.582 - 5.018)	3.262 ± 0.529 (1.835 - 5.100)

Mean ± SEM; range indicated in parentheses; [ ] = n of pts.
Date were rank ordered; analysis of variance was used to determine significance.
Comparisons: B vs BI, a = $P < 0.01$, b = $P < 0.001$.
B vs BIC, c = $P < 0.01$, d = $P < 0.001$.
BI vs BIC, e = $P < 0.01$, f = $P < 0.001$.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OG 1841	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	080
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procode with Security Classification Code)* (U) Assessment of Thyroid Hormone Kinetics in Thermally Injured Patients

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 08	Cont	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	0.4	20
FISCAL YEAR CURRENT 83	0.4	25

19. RESPONSIBLE DOD ORGANIZATION
NAME:* US Army Institute of Surgical Research
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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TELEPHONE: 512-221-5561
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME: POC: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procode EACH with Security Classification Code)
(U) Thyroxine; (U) L-triiodothyronine; (U) L-reverse-T_3; (U) Kinetics; (U) Burn Patients

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procode text of each with Security Classification Code.)

23. (U) To assess metabolic clearance rate and production rate of thyroxine (T_4), triiodothyronine (T_3) in burned soldiers, and to assess the relationship of TSH and cortisol, as well as the effect of other clinical complications of burn injury on thyroid hormone kinetics.

24. (U) Thyroid hormone kinetics were originally assessed in patients with large burns following bolus injection of isotopically labelled thyroid hormones. It has become clear from other work that many factors which influence the course of recovery or death from burn injury may themselves alter thyroid hormone concentrations and kinetics. Therefore, the clinical course of a large number of patients is being followed longitudinally with special attention to medications, sepsis, nutrition, level of consciousness, episodes of surgery, survival vs. nonsurvival, and concentrations of thyroid hormones, TSH, and cortisol. TSH and cortisol are included because they have profound influences on thyroid hormone kinetics.

25. (U) 8110 - 8209. Initial data in burn patients suggested a reduced half-life of T_4 and T_3, and reduced T_3 production, but apparently normal or increased T_4 production. Before obtaining further direct kinetic data, it is necessary to better understand some of the factors mentioned above which might perturb thyroid economy. To date, the clinical courses of 170 patients have been recorded and prepared in

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

a specially designed format for computer analysis which will include our assay results of six different hormones at different time points in each patient. Most of the data have been entered into our PDP 1170 computer.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: ASSESSMENT OF THYROID HORMONE KINETICS IN THERMALLY INJURED PATIENTS: ALTERED TRANSPORT BINDING OF T_4 AND T_3 IN BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1981 - 30 September 1982

Investigators:

George M. Vaughan, M.D., Major, MC
Leonard G. Seraile, M.S.

Reports Control Symbol MEDDH 288 (R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: ALTERED TRANSPORT BINDING OF T_4 AND T_3 IN BURNED SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1981 - 30 September 1982

Investigators: George M. Vaughan, M.D., Major, MSC
Leonard G. Seraile, M.S.

Reports Control Symbol MEDDH 288 (R1)

Burn patients have low levels of T_4 and T_3 and low values that estimate the free concentrations of these hormones whether based on the in vitro T_3 uptake (FT_4I and FT_3I) or the dialyzable fraction (FT_4 and FT_3). Covariance and multiple regression analyses indicate that the free indices (FT_4I and FT_3I) are lower than expected for the FT_4 and FT_3 respectively, resulting from less elevation of the T_3 uptake (T_3U) than of the dialyzable fractions (%D). This discrepancy may result from a binding inhibitor in burns that reduces hormone binding not only to transport serum proteins, but also to the charcoal of the T_3U test. The potential role of reduced concentrations of transport proteins in burns awaits further investigation. Altered transport binding may have an influence on thyroid hormone kinetics. Other clinical factors, such as TSH and cortisol concentrations and various clinical variables may also have an influence on kinetics. Such factors and variables are being studied longitudinally in burn patients, and data from 170 patients have been recorded and are being entered into our computer.

Thyroxine
L-triiodothyronine
L-reverse-T_3
Kinetics
Burn Patients

ALTERED TRANSPORT BINDING OF T_4 AND T_3 IN BURNED SOLDIERS

INTRODUCTION

Like other forms of non-thyroidal illness (NTI), burn injury causes reduced blood concentrations of total tetraiodothyronine (T_4) and triiodothyronine (T_3). Many factors may contribute to this phenomenon, including altered hormone handling, production, or transport binding. In addition, all of these factors may be influenced by levels of TSH and cortisol, and by clinical variables such as burn size (TBS), postburn day (PBD), operations, sepsis, dietary intake, and other variables. All these variables may influence the final circulating concentrations of hormones. Therefore, before addressing whether hormone disposal and production are altered, we are studying the relationships of the above variables with hormonal concentrations in order to better understand what factors are important to control, exclude, or utilize to clearly separate groups of patients in future studies of kinetics. To date, the clinical courses of 170 patients have been recorded and prepared in a specially designed format for computer analysis which will include our assay results of six different hormones at different time points in each patient. Most of the data have been entered into our PDP1170 computer.

In addition, we have analysed transport binding of T_4 and T_3 in a preliminary study of a group of unselected burn patients and uninjured controls by two different methods.

METHODS

For the patients and controls whose characteristics are shown in figures 1 and 2, we sampled serum for determination of total T_4 and T_3 by radioimmunoassay (kits from Diagnostic Products). In addition, in vitro charcoal T_3 uptake (T_3U, Diagnostic Products), and the dialyzable fractions (%D, Nichols Laboratories) for T_4 and T_3 were determined in serum aliquots. Thus, for each hormone, besides the total concentration (T_4,T_3), there are two assessments of transport binding: the percent of added ^{125}I-T_3 tracer that accumulates on the charcoal matrix after incubation with the serum sample (T_3U) and the percent of ^{125}I tracer added as T_4 or T_3 that is free or dialyzable (%D) at equilibrium. Since T_4 and T_3 are bound principally by the same plasma proteins, the T_3U is inversely proportional to the test sample's ability to bind either T_4 or T_3. The product of the T_3U (divided by the T_3U of a normal sample provided in the kit) and total T_4 or T_3, therefore, corrects the T_4 or T_3 to a free index (FT_4I or FT_3I, respectively) that should be proportional to free concentrations of T_4 or T_3 respectively. The %D for each hormone multiplied by the respective total hormone concentration gives the free concentration of T_4 or T_3 (FT_4 or FT_3).

FIGURE 1.

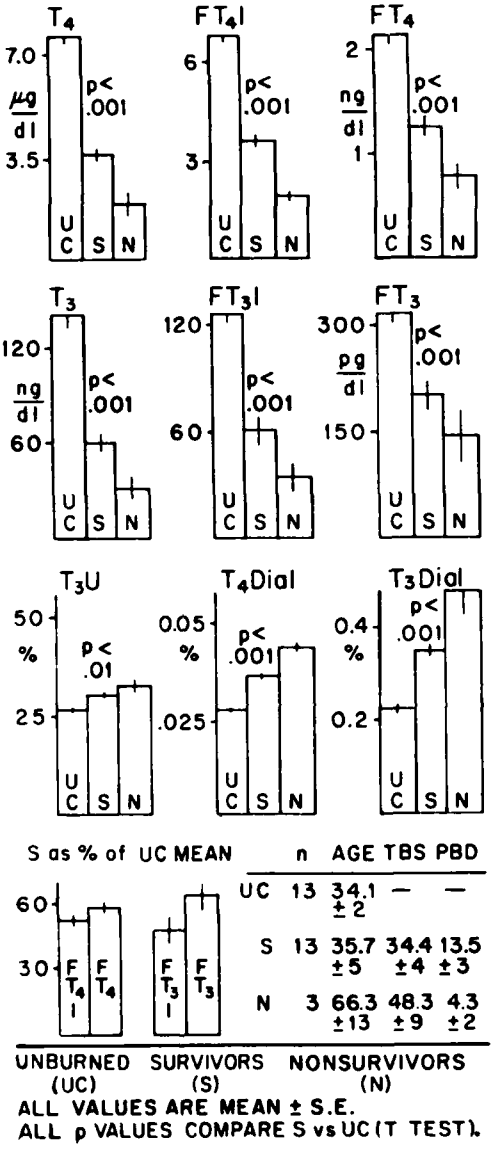

Figure 1. T_4, T_3, free hormonal indices (FT_4I, FT_3I), free concentrations (FT_4, FT_3), in vitro T_3 uptake (T_3U), and dialyzable fraction (Dial) in serum of burn patients and normal controls. Age is in years. TBS, total burn size as % body surface area. PBD, postburn day. Five burn patients had two samples, and the mean between the two for each determination was used in the calculations. The nonsurvivors were not included in the statistical analyses because there were only three of them.

FIGURE 2.

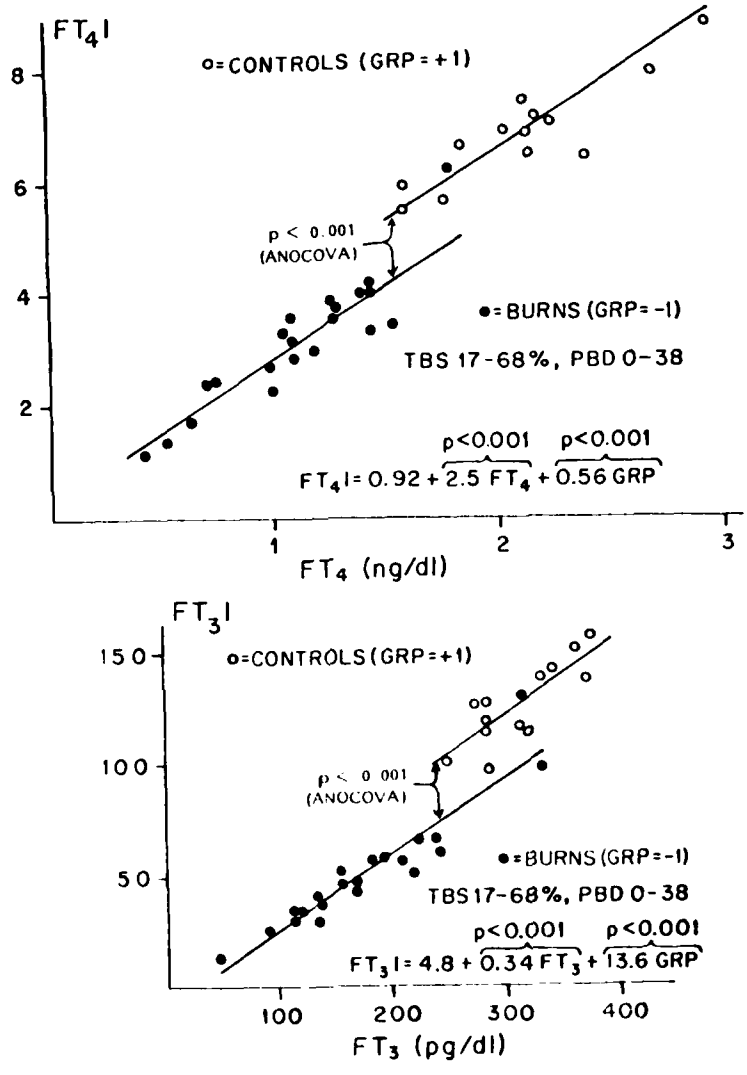

Figure 2. Correlations between free indices (FT_4I, FT_3I) and dialyzable free concentrations (FT_4, FT_3) in the patients and subjects of Figure 1. Analyses of covariance (ANOCOVA) revealed no differences in slope between groups but positional differences as indicated. The regression lines were determined by the multiple regression analyses including group (GRP) as a variable and the relevant equations are above the abscissae. TBS, total burn size as % body surface area. PBD, postburn day. Values from all individual samples are entered into the analyses, including those from the nonsurvivors.

RESULTS

Figure 1 shows that burn patients have suppressed T_4, FT_4I, FT_4, T_3, FT_3I, and FT_3. Whereas T_3U was elevated in these patients, %D^4 (indicated as "Dial" in Figure 1) for T_4 and T_3 were more markedly elevated. Comparison of the free indices with respective free concentrations (Fig 2) shows that for both hormones, the free index is correlated with the free hormone concentration within burns and controls. For each hormone, the slopes were not different between groups but the FT_4I intercepts (or positions of the best-fit lines for each group with a common slope) are significantly different. That is, as the multiple regressions also indicate, the burn patients have a lower free index than predicted by the dialyzable free concentration.

DISCUSSION

The low T_4, T_3, FT_4I, FT_3I, FT_4, and FT_3 in burn patients confirm previous findings from this institute (1,2). However, previous studies (2,3) utilized regressions of free index with free hormone only in burn patients to substantiate the validity of using the free indices to describe changes of free concentrations in such patients. The present study, including non-burned subjects, confirms this approach, because in only one (FT_4I) or two (FT_3I) samples in burns was there an overlap into the normal range, and in these same samples the dialyzable free hormone concentrations also overlapped (Fig. 2).

Although both FT_4I and FT_4 by dialysis are reportedly suppressed in most NTI (4,5), others have expressed concern about misleadingly

1. Becker RA, Wilmore DW, Goodwin CW Jr, Zitzka CA, Wartofsky L, Burman KD, Mason AD, and Pruitt BA: Free T_4, Free T_3, and Reverse T_3 in critically ill, thermally injured patients. J Trauma 20: 713-721, 1980.

2. Vaughan GM, and Becker RA: Thyroid hormones and catecholamines in burn patients: a hypermetabolic low T_3 syndrome. In Ziegler MG, and Lake CR (Eds.) Norepinephrine. Baltimore: The Williams and Wilkins Company, 1982 (in press).

3. Becker RA, Vaughan GM, Ziegler MG, Seraile LG, Goldfarb WI, Mansour EH, McManus WF, Pruitt BA Jr, and Mason AD Jr: The hypermetabolic low triiodothyronine syndrome of burn injury. Crit Care Med, 1982 (in press).

4. Slag MF, Morley JR, Elson MK, Labrosse KR, Crowson TW, Nuttall FQ, and Shafer RB: Free thyroxine levels in critically ill patients. JAMA 246: 2702-2706, 1981.

5. Melmed S, Geola FL, Reed AW, Pekary AE, Park J, and Hershman JM: A comparison of methods for assessing thyroid function in nonthyroidal illness. J Clin Endocrinol Metab 54: 300-306, 1982.

low FT_4I as an index of dialyzable FT_4 concentrations that may be normal or elevated in some patients with NTI (6,7). The present results indicate that in burns, two elements determining the FT_4I can be distinguished. One is the FT_4, which allows use of the index to assess relative suppression of FT_4 among burn patients, and the other is the status of having a burn injury and/or being treated for it, which determines that FT_4I is lower than predicted based on the dialysis result. The same things hold true for FT_3I and FT_3.

It is of interest that burn injury also results in elevation of the %D for T_4 and T_3 and, further, that this reduced transport binding is nevertheless associated with reduced FT_4 and FT_3 concentrations. This indicates that besides affecting transport binding, burn injury has some other effects on thyroid hormone formation and/or degradation.

Observation of reduced transport binding in other NTI led to a search for an inhibitor of binding to thyroid hormone binding proteins (8-10). The nature of the inhibitor, suspected in up to 74% of non-burn NTI patients in the literature based on binding studies, is not yet clear. The present increased %D in burn patients could be explained either by such an inhibitor or by reduction in circulating binding proteins.

In spite of the correlation of FT_4I with FT_4 in our study, we have also demonstrated that the FT_4I in burns is lower than expected for its relationship with FT_4 in controls. Such a discrepancy has been noted in the literature in other types of NTI, as mentioned above, and is probably not simply a result of reduced protein binding of hormone.

6. Chopra IJ, Van Herle AJ, Chua Teco GN, and Nguyen AH: Serum free thyroxine in thyroidal and nonthyroidal illnesses: a comparison of measurements by radioimmunoassay, equilibrium dialysis, and free thyroxine index. J Clin Endocrinol Metab 51: 135-143, 1980.

7. Chopra IJ, Solomon DH, Hepner GW, and Morgenstein AA: Misleadingly low free thyroxine index and usefulness of reverse triiodothyronine measurement in nonthyroidal illnesses. Ann Internal Med 90: 905-912, 1979.

8. Chopra IJ, Chua Teco GN, Nguyen AH, and Solomon DH: In search of an inhibitor of thyroid hormone binding to serum proteins in nonthyroid illnesses. J Clin Endocrinol and Metab 49: 63-68, 1979.

9. Chopra IJ, Solomon DH, Chua Teco GN, and Eisenberg JB: An inhibitor of the binding of thyroid hormones to serum proteins is present in extrathyroidal tissues. Science 215: 407-409, 1982.

10. Oppenheimer JH, Schwartz HL, Mariash CN, and Kaiser FE: Evidence for a factor in the sera of patients with nonthyroidal disease which inhibits iodothyronine binding by solid matrices, serum proteins, and rat hepatocytes. J Clin Endocrinol and Metab 54: 757-766, 1982.

Reduced serum binding elevates the T_3U (% tracer bound to charcoal) which when multiplied by the T_4 corrects it to a higher FT_4I, offsetting the reduction of total T_4 that results from decreased binding (11). This yields an FT_4I that is proportional to the FT_4 by dialysis. That is, in samples with only reduced protein binding, T_3U and % D are elevated proportionately, so that the respective products with total T_4 (FT_4I and FT_4) yield values corrected proportionately upward compared to the values for samples with normal binding. Since there is a slight but significant discrepancy for burn patients (with less elevation of T_3U than % D and lower FT_4I than expected from the FT_4), we suspect the presence of a factor that inhibits the binding of hormone not only to serum proteins but also to the charcoal of the T_3U test. Oppenheimer et al. (10) proposed just such an inhibitor in the serum of non-burned NTI patients. This suggests that the reduced hormone binding in burns is not simply a result of reduced concentration of binding proteins.

Thus, burn patients are similar to other NTI patients in that there is a suppression of T_4, T_3, FT_4I, FT_3I, FT_4, and FT_3, and an increase in the % D. Though there is a discrepancy between the FT_4I and FT_4 as in other NTI, in burn patients this does not preclude interpreting a reduced FT_4I as a reduction in FT_4. Such an interpretation appears more subject to error in other forms of NTI. There appears to be a binding inhibitor in the serum of burn patients, also reported in other NTI. What role reduced levels of binding proteins plays in the reduced binding, as well as the potential role of the injury as separate from therapeutic modalities await further studies including development of an animal model.

11. Ingbar SH, and Woeber KA: The thyroid gland. In Textbook of Endocrinology. VI Ed. Williams RH (Ed.). Philadelphia: W.B. Saunders Company, 1981, pp 117-247.

PRESENTATIONS AND PUBLICATIONS

Vaughan GM, Becker RA, Ziegler MG, Allen JP, Pruitt BA Jr, and Mason AD Jr: Relationships of cortisol, thyroid hormones and catecholamines to the hypermetabolism of burn injury. Presented in part to the VI International Congress on Burns, International Society for Burn Injuries, San Francisco, California, 2 September 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0G 1510	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	H. TERM	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	083
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procode with Security Classification Code)* (U) The Release of Mast Cell Mediators in the Thermally Injured Rat: A Preliminary Assessment for Study of Mast Cell Mediators in the Injured Soldier

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine and 012600 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 01	82 07	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	0.8	22
FISCAL YEAR CURRENT 83	0.0	0

19. RESPONSIBLE DOD ORGANIZATION
NAME:* US Army Institute of Surgical Research
ADDRESS:* Ft Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL
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20. PERFORMING ORGANIZATION
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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TELEPHONE: 512-221-2968
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

POC: DA

22. KEYWORDS (Procode EACH with Security Classification Code)
(U) Rat Model; (U) Burns; (U) Mast Cells; (U) Histamine; (U) Mediators; (U) Leukocytes

23. TECHNICAL OBJECTIVE.* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procode text of each with Security Classification Code.)

23. (U) The quantity of circulating mast cell mediators released during the early postburn period in the rat will be determined. The effect of such quantities of mediators on the immune response will be evaluated. This data will assist in formulation of pharmacologic approaches to modulation of edema and altered host defenses in the burned and injured soldier.

24. (U) Rats will sustain thirty percent total body surface area (TBSA) burns of either partial or full thickness depth or 30% partial plus 30% full thickness burn. In order to evaluate susceptibility to infection partial thickness wounds in rats with 30% or 60% TBSA burn will be inoculated with Pseudomonas aeruginosa strain 59-1244. Mortality, the quantity of neutrophils in the circulation and wounds will be evaluated. Sampling of blood via a subclavian catheter will be performed during the early post burn period.

25. (U) 8110 - 8209. Studies of rats that have had their mast cells degranulated by Polymyxin B injection show that systemic histamine release after partial thickness burn is decreased compared to controls, but edema formation is the same as in controls. Even though the Polymyxin B treated rats had almost a 90% decrease in mast cells, their

*Available to contractors upon originator's approval

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tissue edema was unchanged compared to control rats. Rats with larger burns have been found to be more susceptible to infection. This increased susceptibility was not due to depressed cardiovascular function, but was associated with a two-fold decrease in neutrophils in the burn wounds. The number of circulating neutrophils was not different from rats with smaller burns, but preliminary work suggests that the function of neutrophils in rats with larger burns may be altered.

TERMINATION REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: THE RELEASE OF MAST CELL MEDIATORS IN THE THERMALLY INJURED RAT: A PRELIMINARY ASSESSMENT FOR STUDY OF MAST CELL MEDIATORS IN THE INJURED SOLDIER. EVIDENCE AGAINST PARTICIPATION OF MAST CELL HISTAMINE IN BURN WOUND EDEMA FORMATION

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE army MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1981 - 27 July 1982

Investigators:

Roger W. Yurt, M.D. Major, MC
Arthur D. Mason, Jr., M.D.
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: THE RELEASE OF MAST CELL MEDIATORS IN THE THERMALLY INJURED RAT: A PRELIMINARY ASSESSMENT FOR STUDY OF MAST CELL MEDIATORS IN THE INJURED SOLDIER. EVIDENCE AGAINST PARTICIPATION OF MAST CELL HISTAMINE IN BURN WOUND EDEMA FORMATION

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1981 - 27 July 1982

Investigators: Roger W. Yurt, M.D., Major, MC
Arthur D. Mason, Jr., M.D.
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Studies of rats that have had their mast cells degranulated by Polymyxin B injection show that systemic histamine release after partial thickness burn is decreased compared to controls, but edema formation is the same as in controls. Even though the Polymyxin B treated rats had almost a 90% decrease in mast cells, their tissue edema was unchanged compared to control rats.

Rat Model
Burns
Mast Cells
Histamine
Mediators
Leukocytes

THE RELEASE OF MAST CELL MEDIATORS IN THE THERMALLY INJURED RAT: A PRELIMINARY ASSESSMENT FOR STUDY OF MAST CELL MEDIATORS IN THE INJURED SOLDIER. EVIDENCE AGAINST PARTICIPATION OF MAST CELL HISTAMINE IN BURN WOUND EDEMA FORMATION

Histamine has been implicated as a mediator of edema formation in injured tissue. That this mediator participates in the inflammatory response to injury is suggested by data showing increased levels of plasma histamine during the time of edema formation after burn which are proportional to depth and size of injury (1). Since the mast cell is the principal source of tissue histamine, the contribution of this cell to edema formation in rats with a standard 30% total body surface area (TBSA) partial thickness burn was investigated. Degranulating the mast cells prior to burn injury evoked no difference in the amount of edema formed compared to that in rats with normal mast cells. That degranulation of mast cells affected histamine concentrations after burn injury was confirmed by the observation of substantially lower systemic levels of histamine in the plasma of this group of rats after burn injury.

MATERIALS AND METHODS

Under pentobarbital anesthesia, rats sustained partial thickness scald burns by a standard method of immersion of 30% TBSA in 95°C water for two seconds. Fluid in the burn wound was calculated from the wet weight of two skin biopsies (three to five grams each) from each rat and the weight of the samples after drying for 72 hours at 75°C and reported as percent water. On the basis of the measurement of burn edema at 12 times between five minutes and eight hours postinjury in 55 rats, the five-minute and fourhour time points were selected for further investigation.

The mast cells of one group of rats were degranulated by a three-day pretreatment with IP Polymyxin B. Blood for plasma histamine determination was drawn into citrate in 200 µl volumes

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1. Yurt RW, Mason AD Jr., and Pruitt BA Jr.: Evidence for Mast Cell Mediator Release in Thermal Injury, in Proceedings of Sixth International Congress on Burns, San Francisco, California, August 29 - September 4, 1982.

from central venous cannulae inserted the day prior to the experiment. Plasma histamine was measured using a double-label radioenzymatic assay (2). Mast cells were enumerated in Giemsa stained sections by counting all vessels and mast cells in 10 HPF.

RESULTS AND DISCUSSION

The number of mast cells identified microscopically four hours postinjury was significantly reduced by Polymyxin B pretreatment in both sham and burned rats compared to saline pretreatment, but edema was not different (Table 1). These data combined with those of two additional experiments showed that Polymyxin B (N = 16) and saline (N = 16) treated burned rats had .197 and 1.58 mast cells/vessel, respectively. Even though the Polymyxin B treated rats had almost a 90% decrease in mast cells, their tissue contained the same percent of water as saline treated controls, 71.05 $\pm$ 0.42 and 71.28 $\pm$ 0.26, respectively. At five minutes after injury, both Polymyxin B and saline pretreated rats developed significant amounts of edema compared to controls ($P < 0.04$). However, there was no significant difference ($P > 0.05$) in percent tissue water content between these groups with increases of 1.66% (N = 6) and 2.23% (N = 6), respectively. That histamine stores were depleted was confirmed by the finding that central venous plasma histamine rose from 8.79 $\pm$ 0.86 to 77.58 $\pm$ 27.28 and 74.22 $\pm$ 32.79 ng/ml at one and two minutes postburn, respectively, in the saline pretreated rats but only from 10.75 $\pm$ 6.18 to 20.33 $\pm$ 14.37 and 19.62 $\pm$ 13.29 at these times in rats pretreated with Polymyxin B. Additional studies showed that the plasma histamine of rats pretreated with saline (N = 6) increased sixfold 30 minutes postinjury, while in Polymyxin B treated rats (N = 5) the increase was only twofold ($P < 0.001$).

CONCLUSION

Significant degranulation of mast cells with depletion of histamine stores does not alter edema formation after thermal injury in the rat.

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2. Shaff RE, Beaven MA: Increased Sensitivity of the Enzymatic Isotope Assay of Histamine: Measurement of Histamine in Plasma and Serum. Anal. Biochem. 94:425-430, 1979.

TABLE 1. Results of pretreatment with saline and polymyxin B in sham-injured and burned rats four hours postinjury

Injury	N	Pretreatment	% Water		Mast cells/vessel	
Sham	5	Saline	64.71 ± 0.24 (SEM)		1.97 ± 0.16	*
Sham	5	Polymyxin B	64.01 ± 0.21		0.70 ± 0.17	
Burn	5	Saline	71.41 ± 0.46	NS	1.67 ± 0.22	**
Burn	5	Polymyxin B	70.62 ± 0.51		0.27 ± 0.05	

*P < 0.01; **P <0.001, one-way ANOV.

PUBLICATIONS

Yurt RW, Mason AD Jr., and Pruitt BA Jr.: Evidence against participation of mast cell histamine in burn edema. Surg. Forum, 33:71-73, 1982.

Yurt, RW, and Pruitt BA Jr.: Burns, in Physical Disabilities and Health Conditions: An Introduction. Umbriet, J., Ed., Charles E. Merrill Publishing Co., Columbus, Ohio, In press.

PRESENTATIONS

Yurt, RW, Mason AD Jr., and Pruitt BA Jr.: Evidence for mast cell mediator release in thermal injury. Sixth International Congress on Burns, International Society for Burn Injuries, September 1982.

TERMINATION REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: THE RELEASE OF MAST CELL MEDIATORS IN THE THERMALLY INJURED RAT: A PRELIMINARY ASSESSMENT FOR STUDY OF MAST CELL MEDIATORS IN THE INJURED SOLDIER. DECREASED WOUND NEUTROPHIL RESPONSE RELATED TO EXTENT OF BURN INJURY IN THE RAT

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1 October 1981 - 27 July 1982

Investigators:

Roger W. Yurt, M.D. Major, MC
Arthur D. Mason, Jr., M.D.
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: THE RELEASE OF MAST CELL MEDIATORS IN THE THERMALLY INJURED RAT: A PRELIMINARY ASSESSMENT FOR STUDY OF MAST CELL MEDIATORS IN THE INJURED SOLDIER. DECREASED WOUND NEUTROPHIL RESPONSE RELATED TO EXTENT OF BURN INJURY IN THE RAT

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1981 - 27 July 1982

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A previous report documents that partial thickness burn wound becomes susceptible to bacterial invasion when the extent of burn injury increases from 30% to 60% of the total body surface area. In addition, as in the human patient, mortality from sepsis is significantly increased in the rats that sustain more extensive injury. In order to assess the pathogenesis of the increased susceptibility to infection in rats with more extensive injury, histologic changes in injured skin consisting of neutrophil margination and mast cell degranulation (first 3 hours) and neutrophil infiltration and additional mast cell degranulation (4 - 8 hours) were determined. When wounds of rats with 30% partial thickness injury were compared to those of rats with additional 30% full thickness injury, there were more than two times as many neutrophils in the wounds of the rats with less extensive injury (3.54 + 0.59 cells/vessel) than in those with larger burns (1.61 + 0.41 cells/vessel) at eight hours after injury. There was no difference in availability of neutrophils to the wound since systemic vascular volume as assessed by the urine output, weight change, or red blood cell count, and the number of

Burns
Rat Model
Infectio
Neutrophils

circulating neutrophils were the same in both groups. These findings suggest that as early as 8 hours after injury there is a decreased wound inflammatory response in rats with larger injury which may partially account for increased susceptibility to infection.

INTRODUCTION

The development of a rat model of burn injury (1) that parallels the clinical situation in human patients in which a predictable, although unpredictable for a given patient, increase in mortality from sepsis occurs with increasing extent of injury (2) has provided a means to evaluate the mechanism of increased susceptibility to infection under control conditions. Although depressed neutrophil chemotaxis (3, 4) and chemiluminescence (5), complement consumption (6), abnormalities in macrophage function (7), distorted dynamics of lymphocyte interaction (8), and circulating factors (9) have been implicated in the pathogenesis

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1. Yurt RW, McManus AT, Mason AD Jr., and Pruitt BA Jr.: Increased Susceptibility to Infection related to Extent of Burn. Invasion of Partial Thickness Burn Wounds by Pseudomonas aeruginosa strain 59-1244. Annual Report 1982; U.S. Army Institute of Surgical Research, In press.
2. Moncrief JA and Teplitz C.: Changing Concepts in Burn Sepsis. J. Trauma 1964; 4:233.
3. Warden GD, Mason AD Jr., and Pruitt BA Jr.: Evaluation of Leukocyte Chemotaxis in vitro in Thermally Injured Patients. J. Clin Invest. 1974; 54:1001-1004.
4. Grogan JB: Suppressed in vitro Chemotaxis of Burn Neutrophils. J. Trauma 1976; 16:985-988.
5. Allen RC and Pruitt BA Jr.: Humoral - Phagocyte Axis of Immune Defense in Burn Patients. Arch. Surg. 1982; 117:133-140.
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of sepsis in the human disease, the variability within and the limited size of the patient population at risk has limited the development of a comprehensive theory relating injury induced perturbation of host defense to morbidity and mortality. Rats with 30% partial thickness burns have been found to be resistant to invasion by Pseudomonas aeruginosa strain 57-1244 and their mortality is low (12.5%), however, when an additional 30% full thickness burn is present the partial thickness wound becomes invaded by the organism more frequently and the mortality increases to 50%.

Since mediators of inflammation are known to be released during the acute post burn period (10) and even full thickness burn injured tissue in the rat becomes resistant to microbial invasion if inoculation with this organism occurs more than 72 hours after injury (11), this investigation focused on the acute post burn period. Based on preliminary observations, the histology of wounds and the circulation and function of neutrophils were evaluated at 4 and 8 hours after injury. Since there appeared to be no difference in cardiovascular volume and number of circulating neutrophils but there were more neutrophils in the wounds of rats with less injury, it is proposed that changes in circulating neutrophils or their microenvironment may partially account for the increased susceptibility to infection in rats with more extensive burn injury.

MATERIAL AND METHODS

Male Sprague-Dawley rats weighing 350-380 grams were used in all experiments. Rats that sustained partial thickness scald burn injury had an area of dorsal skin equal to 30% of the total body surface area exposed to 95°C water for 2 seconds through a mold (12). Additional 30% full thickness burn or sham injury was caused by exposure of the ventral surface under the same conditions except that sham injury was performed without exposure to water. Rats that sustained 30% and 60% surface area burns received 15 cc

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10. Yurt RW, Mason AD Jr., and Pruitt BA Jr.: Mast Cell Mediator Release after Thermal Injury. Annual Report, 1981: U.S. Army Institute of Surgical Research, Fort Sam Houston, Texas.

11. Walker HL, Mason AD Jr., and Raulston GL: Surface Infection with Pseudomonas aeruginosa. Ann. Surg. 1964: 160: 297-305.

12. Walker HL and Mason AD Jr.: A Standard Animal Burn. J. Trauma 1968; 8:1049-1051.

received 15 cc and 30 cc of 0.15 saline, respectively, by IP injection at the time of injury. Depth of injury in surviving rats was confirmed by clinical evaluation of wounds at 2 - 4 weeks after injury. Central venous indwelling catheters were placed via the right jugular vein on the day prior to use as previously described (10). Anesthesia prior to burn injury consisted of 25 mg/kg Pentobarbital IP and prior to cannulation consisted of 0.05 ml Innovar injected I.M.

Skin biopsies were taken after Pentobarbital anesthesia by sharp dissection and fixed in 10% buffered formalin. Depth of injury was confirmd by evaluation of hematoxylin and eosin stained sections and all neutrophils, vessels, and mast cells were enumerated in 10 high power fields (later experiments, 30 high power fields) at 450-fold magnification in Giemsa stained sections. Mast cells that had a decrease in intracellular granules with associated granules in the interstitial space were counted as degranulated cells. Cell counts and differentials were performed by standard methods on a ZBI-Coulter Counter and blood smears, respectively. One way analysis of variance was used to determine significance. Linear regression analysis was performed on a T1-59 desk calculator.

RESULTS

In two preliminary experiments neutrophil infiltration and mast cell response were determined in skin biopsies from 5 rats with sham injury and from groups of 5 rats each at 30 minutes, 1, 2, 3, 4, 6, or 8 hours after 30% partial thickness burn injury. Since varying amounts of edema during this time affect the tissue area evaluated microscopically, vessels as well as neutrophils and mast cells were enumerated in 10 high power fields and cell counts were expressed as cells per vessel. There was a progressive increase in marginating neutrophils during the first 3 hours after injury. By 4 hours infiltration of neutrophils became prominent and at 8 hours after injury the majority of the neutrophils were found in the tissue rather than in the vessels (Figure 1). An increase in the percent of mast cells that were degranulated was seen as early as 30 minutes after injury and remained the same over the ensuing five and one-half hours (Figure 2). However, at 8 hours after injury an impressive inflammatory response with large numbers of neutrophils and heightened mast cell degranulation was found. In some cases the inflammatory response varied with regard to intensity within individual biopsies and, therefore, all later biopsies were evaluated by counting 30 high power fields.

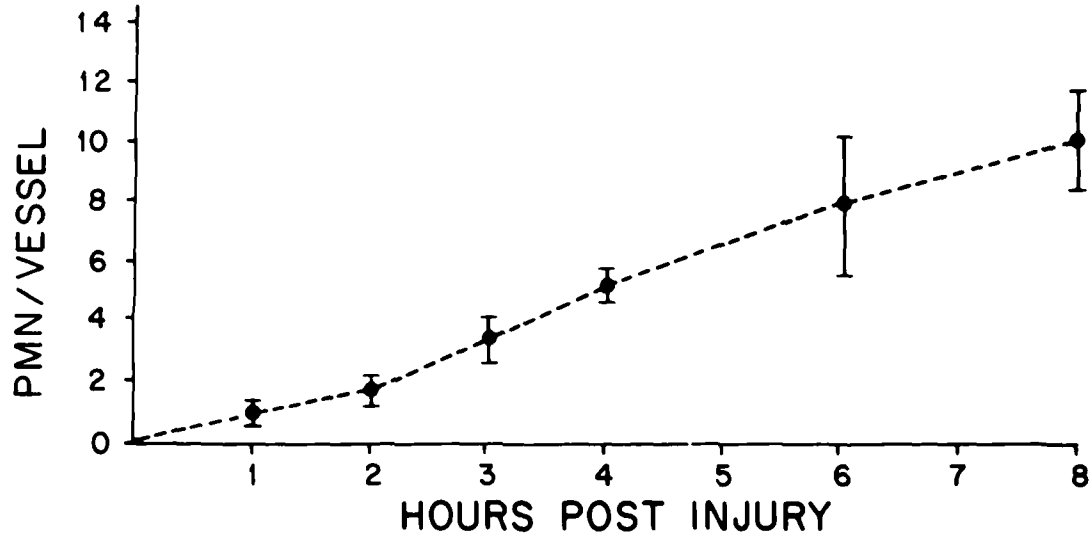

Fig. 1. Neutrophil margination and infiltration after 30% TBSA partial thickness burn.

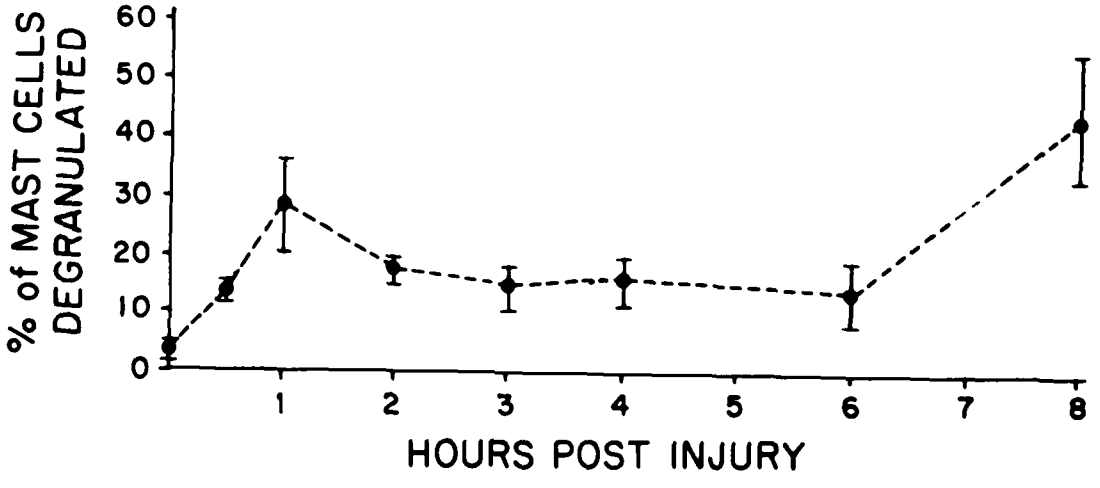

Fig. 2. Mast cell degranulation after 30% TBSA partial thickness burn.

In order to ascertain the relationship between the accumulation of neutrophils in the wound and the number of circulating neutrophils, neutrophil counts were determined on blood drawn from each unanesthetized and unrestrained rat through central venous cannulae at various times after 30% partial thickness burn (N = 5) or sham (N = 5) injury. The number of circulating neutrophils was found to increase in parallel (r^2 = . 984, $p < .001$) with the number of cells in the wounds in the previous experiment (Figure 3).

Based on these preliminary experiments, the 4 and 8 hour times after injury were selected to compare the inflammatory responses in the partial thickness wounds of rats with either 30% partial thickness injury or this injury plus additional 30% full thickness burn. At 4 hours after injury, there were no significant differences between the 30% (N = 5) and 60% (N = 5) burned rat's partial thickness wounds with regard to the number of neutrophils per vessel, the number of mast cells per vessel, or the percent of mast cells degranulated (Table 1). However, by 8 hours after injury the wounds of the 30% burned rats had 4 times more neutrophils per vessel than the wounds of the 60% burned rats. The number of mast cells and percent degranulated mast cells was not significantly different in these groups. Nevertheless, at this time there was a linear correlation, not seen at 4 hours, between the number of neutrophils and the number of degranulated mast cells (r^2 = .61, $p < .005$), suggesting that at this time both cell types were contributing to the inflammatory response in a parallel fashion. To confirm the finding of fewer neutrophils in the wounds of rats sustaining 60% injuries an additional 5 rats were evaluated in each group at 8 hours after injury. Again there were more neutrophils per vessel in the partial thickness wounds of rats with 30% as compared to 60% burn. The combined data from all 8 hour experiments showed that there were more than two times as many neutrophils in the 30% compared to the 60% group ($p < .02$) with the wounds of 30 and 60% burned rats containing 3.54 ± 0.59 and 1.61 ± 0.42 neutrophils per vessel, respectively.

Although previous work suggested that the 30% partial thickness wounds do not convert to full thickness injury in the presence of additional 30% full thickness injury (1), the possibility that the reduced neutrophil migration into the partial thickness wounds of rats with 60% burn could be due to comprised circulation during the acute post burn period remained. Therefore, the urine output and weight changes in the pre and post burn period were evaluated in 30% partial thickness (N = 5) and 30% partial thickness plus 30% full thickness (N = 5) injured rats. Rats were placed in metabolic cages for the 4 days prior to injury and daily urine output and weights were measured. In addition during the immediate post burn period urine output was measured at 4, 8 and 24 hours and the subsequent 3 days after injury. There was no difference in

TABLE 1. Time Related Inflammatory Cell Changes After 30% and 60% Burns in Rats

Injury	Time of Biopsy	PMN/Vessel (± SEM)	Mast Cell/Vessel (± SEM)	% Mast Cells Degranulated (± SEM)
30% **	4 Hours	1.36 ± 0.32	0.96 ± 0.10	16.0 ± 5.10 } NS
60%	4 Hours	1.78 ± 0.36	0.86 ± 0.09	31.4 ± 9.60 } NS
30%	8 Hours	3.78 ± 0.92 } *	1.39 ± 0.14	45.1 ± 14.50 } NS
60%	8 Hours	0.87 ± 0.12 } *	1.20 ± 0.09	21.8 ± 11.40 } NS

* P = 0.025

** 30% Partial Thickness, 60% = 30% Partial Thickness plus 30% Full Thickness

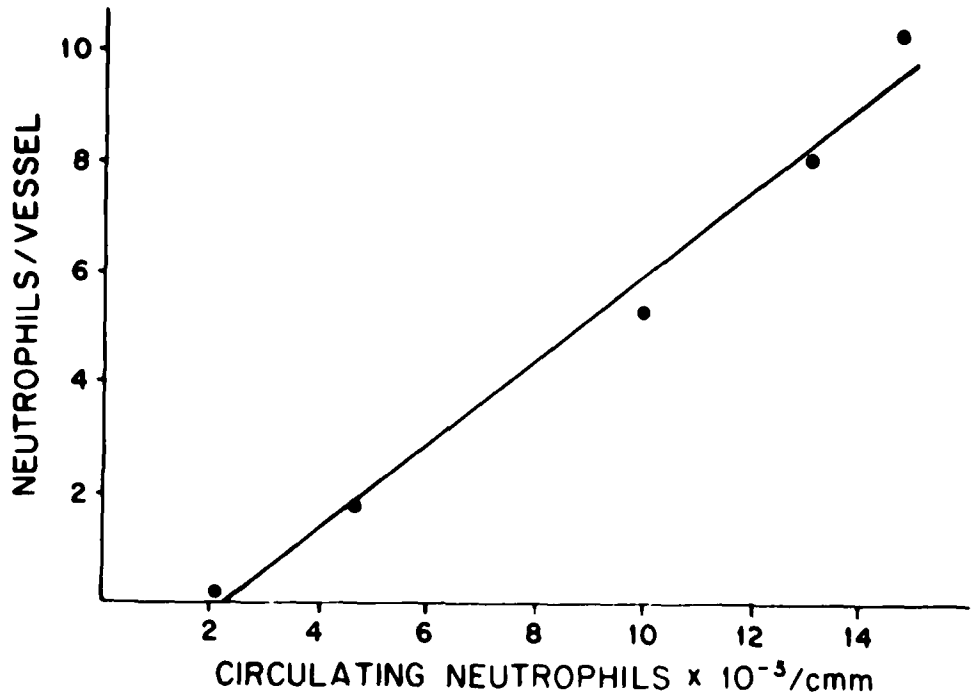

Fig. 3. Relationship between circulating and wound neutrophils in rats after 30% partial thickness burns at various times after injury.

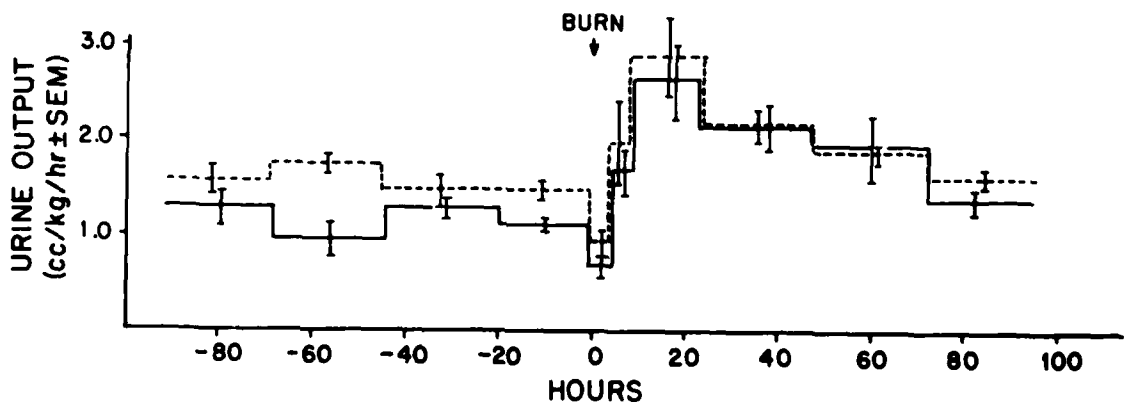

Fig. 4. Effect of 30% partial thickness (------) and 30% partial plus 30% full thickness (———) burn on urine output of rats.

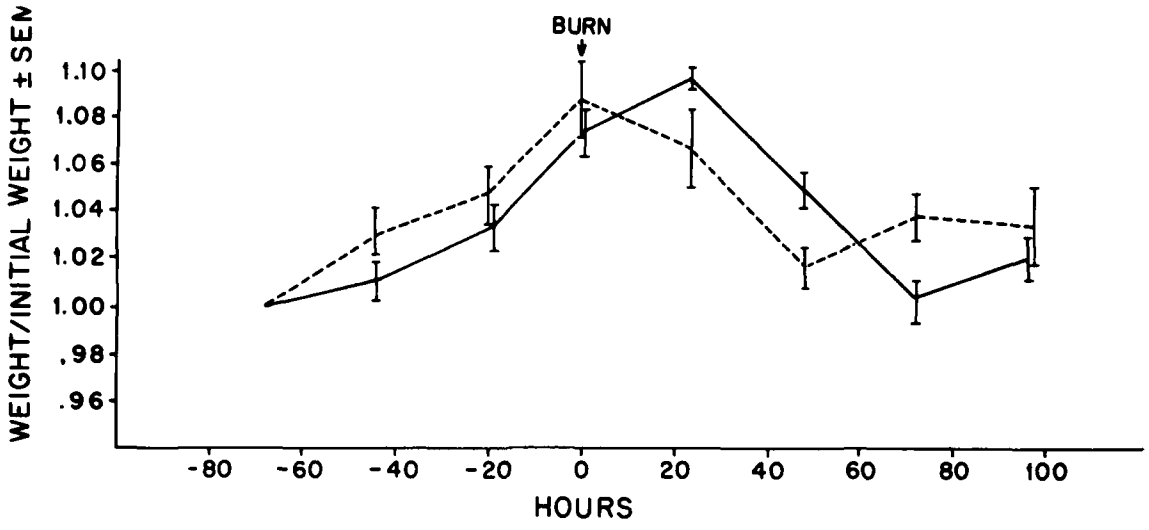

Fig. 5. Effect of 30% partial thickness (------) and 30% partial plus 30% full thickness (———) burn on body weight of rats.

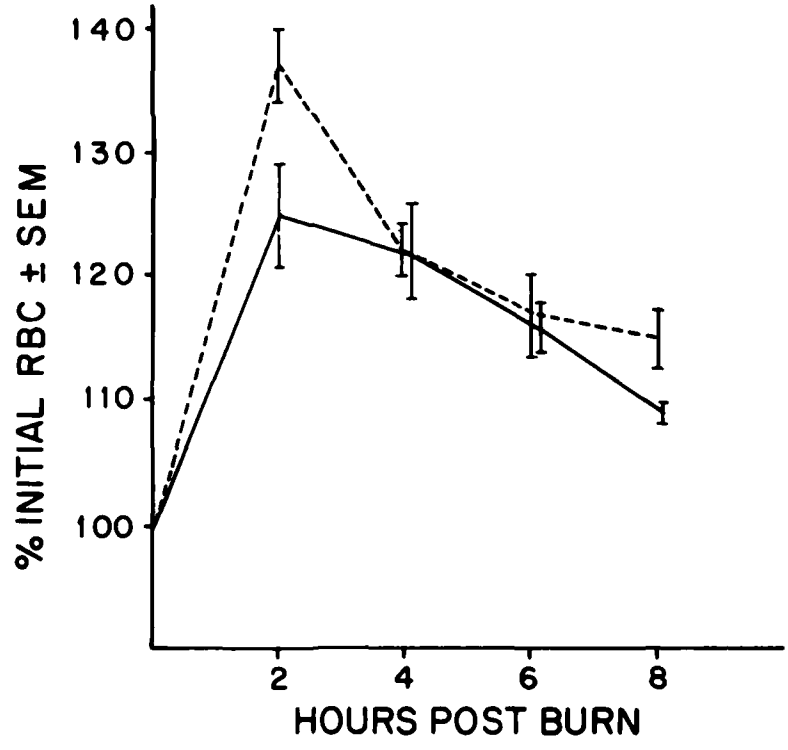

Fig. 6. Time related increase in red blood cell count in rats after 30% partial thickness (------) and 30% partial plus 30% full thickness (———) burn.

in urine output expressed as cc/kg/hr between the two groups (Figure 4). Although the urine output dropped during the 4 hours immediately post injury in both groups, no rat had a urine output of less than 0.35 cc/kg/hr at anytime. From this time on, urine output was above normal in both groups reaching a maximum at the end of the first 24 hours post burn and returning to preburn levels at 4 days after injury. The weight changes in both groups of rats were similar over the time studied (Figure 5).

These data suggested that there was not an appreciable difference between the two groups with regard to systemic circulation. This was supported by an additional study of the change in red blood cell count in rats with 30% partial thickness (N = 5) burns. Erythrocyte counts in serial samples drawn through a central venous cannula in each rat showed no significant difference between groups prior to or at 2, 4, 6, and 8 hours after injury (Figure 6).

Since it appeared that differences in systemic circulation could not account for the depressed neutrophil response in the wound and a correlation had been found between the accumulation of neutrophils in wounds and increase in circulating neutrophils (Figure 3) in preliminary experiments, serial circulating neutrophil counts were determined after these injuries. There was no difference between the mean neutrophil counts (blood drawn serially from central venous cannulae) prior to and at 2, 4, 6, and 8 hours after injury in the 30% partial (N = 5) and the 30% partial plus 30% full (N = 5) thickness burned rats (Figure 7).

DISCUSSION

The time dependent development of the acute inflammatory response in the wounds of rats with 30% partial thickness burns was defined, based on changes in tissue mast cells and neutrophil margination and infiltration. After an early phase of mast cell degranulation and neutrophil margination (30 minutes to 4 hours), neutrophil infiltration became more prominent, as did mast cell degranulation (Figure 1). The early phase, seen histologically, follows and overlaps with the time when systemic mast cell mediator release occurs in such injury (10). This finding is consistent with the hypothesis that mast cell mediators contribute to the development of the acute inflammatory response in injured tissue (13).

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13. Yurt RW: Role of the Mast Cell in Trauma, in Dineen, P. and Hildick-Smith, G (eds): The Surgical Wound, Philadelphia, Lea & Febiger, 1981, pp. 37-62.

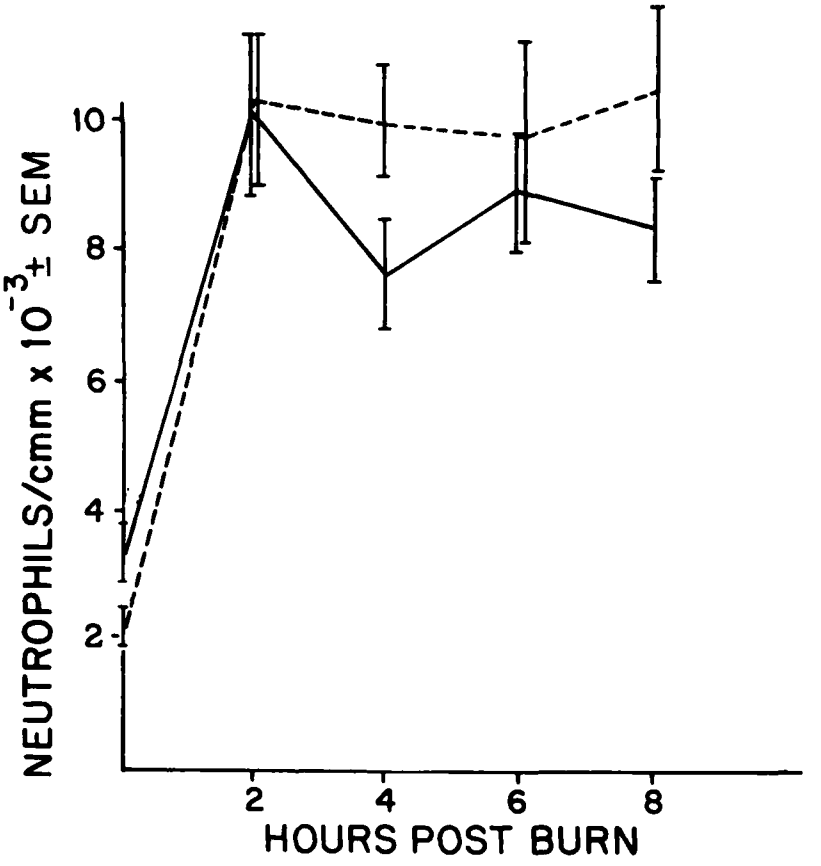

Fig. 7. Time related change in circulating neutrophil counts in rats after 30% partial thickness (------) and 30% partial plus 30% full thickness (———) burn.

During the later phase of inflammation a correlation between the number of neutrophils and degranulated mast cells was observed. This finding is consistent with known mechanisms of interaction between these cells in which the neutrophil chemotactic factor of the mast cell contributes to neutrophil infiltration in inflammation (14) and cationic proteins of neutrophils cause additional mast cell degranulation (15). The finding that the number of circulating neutrophils correlates directly with the number of neutrophils infiltrating the wound of rats with 30% partial thickness burns suggest a passive mechanism of neutrophil accumulation in the wound (Figure 3). Such is not the case, however, since the number of tissue neutrophils varied with extent of injury (Table 1) while the number of circulating cells did not change (Figure 7).

When partial thickness wounds were evaluated in rats with 30% partial thickness or 30% partial thickness plus 30% full thickness injury, the wounds from rats with the smaller injury contained from 2 to 4 times more neutrophils at 8 hours after burn (Table 1). At this time a correlation between the number of wound neutrophils and degranulated mast cells was also found. The mechanism of decreased inflammatory cell infiltration in the wounds of rats with larger injury did not appear to be related to the number of neutrophils available at the wound site, since cardiovascular function, as assessed by urine output (Figure 4), weight change (Figure 5), and red blood cell count (Figure 6), was not different and the number of circulating neutrophils per cubic milliliter was the same (Figure 7). Based on these data, a likely alternative explanation of the difference in neutrophil accumulation is a difference in neutrophil response.

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15. Ranadive NS and Cochrane CG: Mechanism of Histamine Release From Mast Cell by Cationic Protein (Band 2) from Neutrophil Lysosomes. J. Immunol. 1971; 106:506.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 5028	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	087
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Role of Lipid Metabolism in Burn Injury

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine and 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 02	Cont	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	0.8	52
FISCAL YEAR CURRENT 83	0.8	64

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME: POC: DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Lipid Metabolism; (U) Fatty Acid Oxidation; (U) Mass Spectroscopy; (U) Burn Injury; (U) Mitochondrial; (U) Gluconeogenesis; (U) Lab Animal

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To evaluate in an animal model the changes in lipid metabolism which have been observed following thermal injury in burned soldiers and to assess the effectiveness of conventional nutritional support in the presence of these alterations.

24. (U) The isolated adipocyte is being used to determine the metabolic response of adipose tissue to various hormonal alterations associated with thermal injury. Changes in tissue lipid composition and metabolic pathways are being investigated using gas chromatography-mass spectroscopy.

25. (U) 8110 - 8209. Initial experiments demonstrated a significant decrease in the ability of epinephrine to stimulate lipolysis in adipocytes from burned animals when compared to unburned controls. Continuing investigation has led to the following results: (1) the phenomenon has been verified through repeated series of experiments; (2) the decreased response exists over a broad range of epinephrine concentrations and cannot be explained by simple shifts in the hormonal dose-response curves of the cells; (3) the alteration is not directly connected to observed decreases in cell size following burning; (4) no changes in lipid composition can be detected in adipocyte triglyceride due to injury; (5) preliminary experiments suggest that the role of inhibitory metabolite production (specifically adenosine) may be significant.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

PROJECT TITLE: THE ROLE OF LIPID METABOLISM IN BURN INJURY - LIPOLYTIC RESPONSIVENESS TO EPINEPHRINE IN ADIPOCYTES

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
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1 October 1981 - 30 September 1982

Investigators:

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Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: THE ROLE OF LIPID METABOLISM IN BURN INJURY - LIPOLYTIC RESPONSIVENESS TO EPINEPHRINE IN ADIPOCYTES

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Period covered in this report: 1 October 1981 - 30 September 1982

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Severe thermal injury is followed by a period of hypermetabolism which is characterized, in part, by increased mobilization of fat and elevated catecholamines. The effect of burn injury on the lipolytic response of adipose tissue to acute epinephrine stimulation was investigated in adipocytes isolated from the epididymal fat of male Sprague-Dawley rats on the 12th postburn day. Adipocytes were incubated in the presence and absence of 10^{-5} M epinephrine; lipolysis was measured as glycerol produced in nmole x 10^6 cells^{-1} x hr^{-1}. Unstimulated rates of lipolysis were indistinguishable in adipocytes from burned and control rats at all times. Stimulated rates of glycerol production, on the other hand, were significantly lower in the burned group. This decreased rate of glycerol production could not be accounted for by the 17-hr fast. In addition to the decreased lipolytic response, cells from the burned group were smaller in size and lower in triglyceride content. The results indicate that, although epinephrine is effective in stimulating lipolytis in adipocytes from burned rats, the magnitude of lipolytic response is reduced when compared to control values.

Adipocytes
Lipolytic stimulation
Glycerol production

THE ROLE OF LIPID METABOLISM IN BURN INJURY - LIPOLYTIC RESPONSIVENESS TO EPINEPHRINE IN ADIPOCYTES

Severe injury is commonly associated with significant alterations in metabolism. In the case of extensive burn injury in humans, metabolism is at first suppressed (1). This suppression is followed by a sustained increase in resting metabolic rate above normal, approaching a 100% increase in patients with extensive burns (1,2). This hypermetabolism maximizes at 10 to 15 days following injury and returns slowly toward normal as healing progresses (1). Among the many characteristics of the hypermetabolic state is an increased mobilization of body fat (1-3), which is reflected in increased serum fatty acid and glycerol concentrations (3,4), increased glycerol turnover (5), increased rate of clearance from the circulation of infused lipid emulsions (6), rapid depletion of body fat deposits (7,8) and fatty acid deficiencies (6). The magnitude of these changes depends on the type and extent of the injury (3,8,9).

1. Wilmore DW, and Aulick LH: Metabolic changes in burn patients. Surg Clin North Am: 1173-1187, 1978.

2. Elwyn DW: Nutritional requirements of adult surgical patients. Crit Care Med 8: 9-20, 1980.

3. Harris RL, Frenkel RA, Cottam GL, and Baxter CR: Lipid mobilization and metabolism after thermal trauma. J Trauma 22: 194-198, 1982.

4. Birke G, Carlson LA, and Liljedahl S-O: Lipid metabolism and trauma. III. Plasma lipids and lipoproteins in burns. Acta Med Scand 178: 337-350, 1965.

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6. Wilmore DW, Moylan JA, Helmkamp GM, and Pruitt BA Jr.: Clinical evaluation of a 10% intravenous fat emulsion for parenteral nutrition in thermally injured patients. Ann Surg 178: 503-513, 1973.

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8. Davies JWL, and Fell GS: Tissue catabolism in patients with burns. Clin Chim Acta 51: 83-92, 1974.

9. Wilmore DW, Long JM, Mason AD Jr., Skreen RW, and Pruitt BA Jr.: Catecholamines: Mediator of the hypermetabolic response to thermal injury. Ann Surg 180: 653-669, 1974.

Changes in the regulatory hormones for lipolysis accompany the increased mobilization of lipid that is observed under these conditions. The concentrations of circulating catecholamines (9-11) and glucagon (12) are elevated early in the postburn course, whereas insulin is reduced (12). Plasma insulin returns to pre-burn values within the first two weeks post-injury (12,13) while glucagon (12,14) and catecholamines (9-11) remain elevated, returning toward normal as wound healing progresses. Serum triiodothyronine (T_3) is decreased following burn injury, changing inversely with catecholamine levels (15).

The goal of these experiments was to investigate adipose tissue function in burned and control animals. Because of the strong lipolytic effects of epinephrine and the candidacy of catecholamines as mediators of the hypermetabolic response (9), chronic elevation of this hormone following thermal injury may result in changes in the ability of adipose tissue to mobilize lipids. This possibility was explored by assessing the ability of isolated adipocytes to respond lipolytically to acute epinephrine stimulation.

MATERIALS AND METHODS

Male Holtzman rats weighing 450-500 grams were housed individually in a 25°C room for one week prior to study. Free access to both food and water was provided at all times. The housing area was maintained on a 10/14 hr light/dark cycle beginning at 6:00 in the morning. All animals were sacrificed at 9:00 a.m. after a 17-hr fast.

10. Aikawa N, Caulfield JB, Thomas RJS, and Burke JF: Postburn hypermetabolism: Relation to evaporative heat loss and catecholamine level. Surg Forum 26:74-76, 1975.

11. Aprille JR, Aikawa N, Bell TC, Bode HH, and Malamud DF: Adenylate cyclase after burn injury: Resistance to desensitization by catecholamines. J Trauma 19:812-818, 1979.

12. Wilmore DW: Carbohydrate metabolism in trauma. Clinics in Endocrinol and Metab 5:731-745, 1976.

13. Wilmore DW, Mason AD Jr., and Pruitt BA Jr.: Insulin response to glucose in hypermetabolic burn patients. Ann Surg 183:314-320, 1976.

14. Wachtel TL, Shuck JM, Schade D, Eaton RP, and Shuck LW: Hyperglucagonemia and hepatic ketogenesis in burned swine. J Trauma 18:248-253, 1978.

15. Becker RA, Vaughan GM, Goodwin CW Jr., Ziegler MG, Harrison TS, Mason AD Jr., and Pruitt BA Jr.: Plasma norepinephrine, epinephrine and thyroid hormone interactions in severely burned patients. Arch Surg 115:439-443, 1980.

In the first series of experiments (Series I), rats were randomly divided into two groups. One group was anesthetized (5 mg sodium pentobarbital/100 grams body weight), shaved and subjected to a 60% total body surface full-thickness, scald burn (16). These animals were resuscitated by an intraperitoneal injection of 30 ml of 0.9% saline. Animals in the remaining group were untreated. Six animals were chosen at random from each group and sacrificed on postburn day 12.

Each animal was treated according to the following procedure. Following sacrifice, the epididymal fat pads were removed and placed in warm Krebs-Ringer phosphate buffer (KRP). The distal portions of the fat pads were minced and digested (17) for 60 min at 37°C in KRP containing 40 mg/ml albumin (KRPA) (Fraction V, Sigma Chemical Corp., Lot 80F07071) and 3 mg/ml collagenase (Type I, Worthington Biochemical Corp., Lot 40K043). Adipocytes were separated from the stromal-vascular elements by filtration through a 105 μ nylon mesh. The cells were then washed, suspended in KRPA and dispensed into separate plastic flasks containing 4.0 ml KRPA. Final cell concentrations ranged from 50,000 to 125,000 cells/ml. Three pairs of samples were incubated at 37°C with gentle shaking. Epinephrine was present in one pair of samples at a final concentration of 10^{-5} M, a dose resulting in maximal stimulation of normal adipocytes (18,19). The second pair contained no hormone and furnished values for determining basal rates. Incubation of these two pairs was halted at the end of 60 min by mixing the samples with 0.05 volume cold perchloric acid (PCA: 50% w/v). The third pair of samples was mixed with PCA as soon as the cells were dispensed into the incubation flasks to provide pre-incubation values. All samples were filtered, and the filtrates were stored at -20°C prior to analysis.

The second series of experiments (Series II) was designed to assess the effect of the 17-hr fast on both basal and stimulated adipocyte glycerol production. Animals were divided into burned and normal groups. The evening prior to the experiment, half of the animals in each group were deprived of food. The burned animals were all sacrificed on postburn day 12. All other experimental conditions were the same as Series I.

16. Walker HL, and Mason AD Jr.: A standard animal burn. J Trauma 8:1049-1051, 1968.

17. Rodbell M: Metabolism of isolated fat cells. I. The effects of hormones on glucose metabolism and lipolysis. J Biol Chem 239:375-380, 1964.

18. Burns TW, Langley PE, Terry BE, Bylund DB, Hoffman BB, Tharp MD, Lefkowitz RJ, Garcia-Sainz JA, and Fain JM: Pharmacological characterizations of adrenergic receptors in human adipocytes. J Clin Invest 67:467-475, 1981.

19. Bukowiecki L, Lupien J, Follea N, Paradis A, Leblanc D, and Leblanc J: Mechanism of enhanced lipolysis in adipose tissue of exercise-trained rats. Am J Physiol 239:E422-E429, 1980.

All PCA filtrates were analyzed for glycerol content by enzymatic spectrophotometric assay using a modified form of Garland and Randle's technique (20). Samples were neutralized and PCA was removed by adding microliter amounts of 10N KOH. Glycerol standards were made up at the time of each experiment and stored with and treated as samples throughout the preparatory steps in the assay procedure.

Glycerol production (nmole x 10^6 $cells^{-1}$ x hr^{-1}) was calculated as the difference in glycerol concentration between the 60-min incubations and the incubations that were terminated at time zero, normalized to the number of cells in the incubation flask. Increases in the rates of glycerol production due to epinephrine were obtained by taking the difference between the stimulated rate of glycerol production and the basal rate.

The number of cells per unit volume was determined for the original cell suspension and corrected for dilution in the incubation medium. Cells which had been fixed in osmium tetroxide (Degussa Corp.) were washed, suspended in 50% glycerol in water and counted under a microscope. This process yielded 100-400 cells per 5 µl aliquot and had a small replicate error (coefficient of variation < 10%).

Cell diameters and triglyceride content were also measured in a third series (Series III) of 8 normal and 8 burned animals. The diameters of 75 randomly chosen osmium-fixed cells per animal were measured microscopically with an eye-piece reticle. The observed diameters described a normal distribution. Triglyceride content was determined as follows: A 1.0 ml aliquot of cells was homogenized in 20 volumes 2/1 chloroform/methanol (C/M). After filtering, the mixture was washed with 0.88% KCl to a final ratio of C/M/H_2O of 1/1/0.75. The upper phase was aspirated and discarded, and the lower phase was washed with 1/4 volume of 1/1 Methanol/H_2O. The lower phase was collected, concentrated and analyzed for triglyceride content by colorimetric assay using Sigma Kit 405.

Statistics were performed by analysis of variance.

RESULTS

It can be observed in Table I that there were no distinguishable differences in basal rates of glycerol production between the normal and burned rats in Series I. Stimulated rates of glycerol production, on the other hand, were significantly decreased in adipocytes from burned animals with respect to normal ($p < 0.01$).

Table II presents the basal and stimulated rates of glycerol production for animals which were either fasted 17 hr or not fasted prior to sacrifice (Series II). There was an increase in basal rate of glycerol production following fasting, which was significant in the burned group. However, the

20. Garland PB, and Randle PJ: A rapid enzymatic assay for glycerol. Nature 196:987-988, 1962.

results show that the overnight fast cannot account for the observed differences in stimulated rates of glycerol production between adipocytes from burned and normal animals.

Table III presents the cell size and triglyceride content data of series III. Measurement of cell diameters of burned rats revealed a significant decrease in size when compared with untreated controls. This decrease in cell size was reflected in a decrease in cell triglyceride content in cells from burned animals. This decrease in cell size and triglyceride content was accompanied by a lower average rat weight in the burned group compared to normal.

DISCUSSION

The role of fat in postburn hypermetabolism is still mostly undefined. Provision of excess glucose does not appear to offset either fat oxidation (2,5,21) or triglyceride breakdown (5) during the hypermetabolic phase of severe injury in humans; whether this is true for burn injury has not been reported. Furthermore, fat supplied in the diet as non-protein calories will offset nitrogen losses in patients with moderate injuries (21), but not in patients with severe burn injuries (22). It is clear that the use of fat for oxidative energy is increased following most injuries (5,6,21), necessitating higher rates of mobilization of free fatty acids from adipose tissue. This elevated lipolysis is associated with high levels of circulating catecholamines, which are evidenced by enhanced excretion rates during the post-injury period (9-11).

Our results show that the ability of adipocytes to respond to 10^{-5} M epinephrine in terms of lipolysis is depressed in burn-injured animals when compared to controls. The position that this depressed lipolytic responsiveness to epinephrine in vitro occupies in the integrated in vivo metabolic reaction to burn injury cannot be deduced from this investigation. The elevated rate of lipolysis observed in vivo is, in theory, attributable to the elevated levels of circulating catetholamines. What our experiments have shown is that epinephrine is still effective in stimulating lipolysis, but that the magnitude of response is decreased in adipocytes from the burned animal when compared with untreated controls. It is possible that the observed reduction in hormonal responsiveness helps to reduce the rate at which fat stores are depleted, prolonging substrate availability.

The effect of adrenergic stimulation on hypermetabolic lipolysis in burn injury has received some attention. Aprille et al. (11) have shown that a single acute exposure to isoproterenol results in equal increases

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22. Long JM III, Wilmore DW, Mason AD Jr, and Pruitt BA Jr: Effect of carbohydrate and fat intake on nitrogen excretion during total intravenous feeding. Ann Surg 185:417-422, 1977.

TABLE I. Glycerol Production (nmole x 10^6 cells^{-1} x hr^{-1})

SERIES I

	BASAL	STIMULATED
NORMAL	99.6 $\pm$ 18.2	5759.4 $\pm$ 307.0
N = 6		
BURN	91.9 $\pm$ 13.8	3414.4 $\pm$ 641.1[a]
N = 6		

[a] $p < 0.01$ burn vs normal.

TABLE II. Glycerol Production (nmole x 10^6 cells^{-1} x hr^{-1})

SERIES II. Fasted vs Nonfasted

		BASAL		STIMULATED	
		NonFast	Fast	NonFast	Fast
NORMAL	$\bar{X}$	18.8	328.8	8260.2	8116.0
	SE	189.8	181.5	438.7	571.3
		N = 3	N = 4	N = 3	N = 4
BURNED	$\bar{X}$	32.6	317.0[a]	3720.6[b]	3084.0[b]
	SE	15.5	33.9	521.0	672.8
		N = 3	N = 5	N = 3	N = 5

[a] $p < 0.001$ Fast vs Non-Fast.

[b] $p < 0.01$ Burn vs Normal.

TABLE III. Cell size and triglyceride content.

	SERIES III	
	Normal	Burn
Cell size (μ)	76.7 ± 1.0	69.3 ± 1.4[a]
Triglyceride ($mg/10^6$ cells)	229.0 ± 12.0	170.0 ± 10.0[a]
Average rat weights (grams)	457.6 ± 2.6	400.6 ± 8.2[a]

[a] $p < 0.01$ burn vs normal.

in adenylate cyclase activity in adipocytes isolated from burned (20% total body surface), sham-burned and normal rats. They also found that, unlike cells from normal animals, adipocytes from burned animals were not desensitized in terms of adenylate cyclase activation following either acute or chronic catecholamine exposure. The conclusion of Aprille's work is that adipose tissue should have a normal response to catecholamines, at least in terms of adenylate cyclase activation, even after prolonged catecholamine exposure such as occurs in the postburn course.

There are several possible explanations for the apparent discrepancy between the results presented in this paper and those of Aprille et al. First, the alterations in the metabolic pathways responsible for the depressed lipolytic response may be distal to the involvement of adenylate cyclase, that is, at the level of protein kinases or hormone-sensitive lipase. Furthermore, increased extent of burn is associated with increased hypermetabolism (9,23), and the degree of hypermetabolism in the burned rat can be affected by the temperature in which the animal is housed (23,24), especially in moderate burns. Therefore, the more extensive burn used in our experiments (60% vs. 20% total body surface), the differing animal age-weight ranges (500 g in our study vs. 250 g) and possible differences in the environment in which rats were housed could contribute to different observations. The fact that Aprille et al. used isoproterenol whereas we used epinephrine should result in only quantitative differences, since both agonists should give pure beta-receptor stimulation in rat adipocytes (25).

Finally, our results show the lipolytic responsiveness to epinephrine in adipocytes from burned animals is significantly less than that seen in untreated animals and cannot be accounted for by overnight fasting. This clearly shows that significant alterations have occurred at the cellular level in adipose tissue that can be attributed to burn injury alone. Whether the decrease in lipolytic response can be explained on the basis of triglyceride depletion is not fully answered by these measurements. What can be deduced is that the store of triglyceride left in the cells is several orders of magnitude greater than that being degraded and released through lipolysis during the one hour incubation of these experiments. Therefore, if the reduction in cell triglyceride content is a factor, it is involved in some way other than simple depletion of a single source pool.

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25. Burns TW, and Langley PE: Lipolysis by human adipose tissue: The role of cyclic 3',5'-adenosine monophosphate and adrenergic receptor sites. J Lab Clin Med 75:983-997, 1970.

In summary, extensive thermal injury is associated with a decrease in the lipolytic response of isolated adipocytes to a given epinephrine stimulation when compared with cells from normal animals. The reduction in response is associated with a decrease in cell size and triglyceride content but does not appear to be due to a simple depletion of triglyceride stores. The most likely candidates are alterations in receptors or receptor-hormone interactions or changes in the cellular enzymes.

PRESENTATIONS/PUBLICATIONS

Strome DR, Goodwin CW Jr, and Mason AD Jr: Decreased hormonal responsiveness in adipocytes following severe thermal injury. Fed. Proc. 41:1741 (Abstract 8600), 1982. (Presented at Federation of American Societies for Experimental Biology Meeting, New Orleans, Louisiana, April 23, 1982.)

Strome DR, Goodwin CW Jr, and Mason AD Jr: Effects of catecholamines on adipocyte metabolism in burn injury. Presented at American Burn Association Annual Meeting, Boston, Massachussetts, 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
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3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
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19. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
NAME:
NAME: POC: DA

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23. TECHNICAL OBJECTIVE.* 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To evaluate the changes that occur in skeletal muscle metabolism after thermal injury and to determine means of reducing mortality due to the severe catabolic state observed in the burned soldier.

24. (U) Using standard differential respirometry techniques, liquid scintillation counting procedures for radioassays, and enzyme concentration and kinetic measurements, the metabolic response of skeletal muscle to burn injury will be delineated.

25. (U) 8110 - 8209. Two initial studies have been completed during this fiscal year. One study showed that muscle undergoes cellular adaptations during the hypermetabolic period after thermal injury. These adaptations include: (1) increased citrate synthase, glutamate-pyruvate transaminase, and, phosphofructokinase activity and (2) increased ability of the muscle to oxidize fat as a substrate for energy production. The findings of this study have been accepted for publication in the journal, Metabolism. The second study completed during this fiscal year was entitled, "Neutral Proteinase activity from thermally injured rats". In this study, it was shown that neutral proteinase activity (not requiring Ca^{+2} for activity) decreased by the

*Available to contractors upon originator's approval

third postburn day. This is an important finding in determining the cause of increased muscle protein catabolism after injury. The results of this study have been submitted to Biochem, Biophys, Acta for publication.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: CHARACTERIZATION OF SKELETAL MUSCLE METABOLISM AFTER THERMAL INJURY

US ARMY INSTITUTE OF SURGICAL RESEARCH
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1 October 1981 - 30 September 1982

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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: CHARACTERIZATION OF SKELETAL MUSCLE METABOLISM AFTER THERMAL INJURY

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

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Male Sprague-Dawley rats that received 60% total body surface, full-thickness, scald burns on the dorsum and abdomen were used in this study. Neutral proteinase and Ca^{+2}-activated neutral proteinase activities were measured in gastrocnemius and soleus muscles at 3 and 21 days after the thermal injury. Neutral proteinase activity decreased significantly in the soleus (33%) and gastrocnemius (32%) muscles on the third postburn day. Ca^{+2}-activated neutral proteinase was unchanged at this time. Neutral proteinase and Ca^{+2}-activated neutral proteinase activity were unaltered at 21 days postinjury. These results may reflect a protein sparing effect on the third postburn day which could be an early intracellular change prior to an increase in selected enzyme proteins during the hypermetabolic phase after thermal injury.

Skeletal muscle metabolism
Burn injury
Neutral protease
Neutral proteinase
Laboratory animal

CHARACTERIZATION OF SKELETAL MUSCLE METABOLISM AFTER THERMAL INJURY

Subcellular muscle proteins are known to turn over at different rates (1,2), but the role of proteinases in skeletal muscle protein degradation is unclear. The best characterized proteinases are the cathepsins, which are lysosomal proteinases that are active over acidic pH ranges (3,4). However, these proteinases are not highly substrate specific and are not generally active within a neutral pH range (5,6). These considerations cast doubt on the role of cathepsins in protein degradation in vivo. Several investigators have identified a proteinase active at alkaline pH in rat muscle (7,8). It has been postulated that this proteinase is of mast cell origin and is essentially the same enzyme identified as mast cell alkaline protease (9). It is doubtful that this proteinase is involved in intracellular muscle protein degradation because of its extracellular location and pH optimum. Neutral proteinases, active at near neutral pH values, have been identified in skeletal muscle (10-13). These

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proteinases are extra-lysosomal, and their proteolytic activity is low in comparison to acid and alkaline proteinases (6). Within this category of proteinases is the calcium-activated neutral proteinase. This proteinase requires calcium ions for activity and degrades the Z-disk of myofibrils specifically (12-13). Neutral proteinases and calcium-activated neutral proteinase, in particular, are likely candidates for a regulatory role in intracellular protein degradation in vivo since they are active at a near neutral pH and show a high degree of substrate specificity.

It was of interest to examine neutral proteinase activity in animals subjected to the trauma of thermal injury since injury is known to cause a negative nitrogen balance and skeletal muscle is believed to contribute to this loss of nitrogen (14,15). A change in muscle protein synthesis and/or degradation after injury might be reflected by altered neutral proteinase activity. In this investigation, total neutral proteinase activity and Ca^{+2}-activated neutral proteinase activity were measured in skeletal muscle from thermally injured animals in order to determine whether these proteinases played a role in the altered protein metabolism previously observed after thermal injury.

METHODS

Animal care and treatment

Male Sprague-Dawley rats were maintained on a diet of Purina laboratory chow and water provided ad libitum and exposed to a 12:12-hour light-dark cycle. The animals were divided into two groups: a sham-control group and a burned group. The animals were burned using the procedure described by Herndon et al. (16). Briefly, this procedure consists of anesthetizing the rat (50 mg pentobarbital/kg), shaving the area to be burned, placing the animal in a body mold which exposes a known percentage of the total body surface (TBS), and scalding the exposed area in water to produce the desired wound depth. In this experiment, the rats (350-370 g) received a 60% TBS burn (30% on the dorsum and 30% on the abdomen). In order to produce a full-thickness wound and minimize damage to underlying tissues, the dorsum was scalded for 9 sec and the abdomen for 3 sec in 98° C water. Saline (30 ml) was given intraperitoneally prior to scalding the abdomen to provide protection to the viscera and to aid in the resuscitation of the animal. The sham-control group was anesthetized and shaved. Animals from each group were sacrificed at 3 and 21 days postinjury. All experiments were performed at the same time of day.

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16. Herndon DN, Wilmore DW, Mason AD Jr: Development and analysis of an animal model simulating the human postburn hypermetabolic response. J Surg Res 25:394-403, 1978.

Tissue sampling and processing

The soleus and gastrocnemius muscles were removed from anesthetized rats on the specified postburn day. The soleus is classified as an intermediate fiber type muscle (intermediate oxidative capacity, low glycolytic capacity), and the gastrocnemius is a mixed fiber type muscle (50% of the fibers are "white," the remainder intermediate and red fibers). These muscles were chosen in order to examine the proteolytic rate in muscles of differing fiber composition. The muscles were dissected free of connective tissue, minced on ice, and weighed prior to dilution for homogenization. A 10% (w/v) homogenate was prepared from each muscle with 150 mM KCl (pH 7.0) using an Ultra-Turrax homogenizer (Tekman Ind., Cincinnati, Ohio). The whole homogenate was stored at -80° C until analysis, at which time all samples were analyzed simultaneously.

Proteinase activity

Neutral proteinase activity was determined for each sample using the assay procedure described by Kar and Pearson (17). Neutral proteinase activity in the absence of Ca^{+2} was determined by subtracting a blank containing no homogenate sample from a tube containing substrate and homogenate sample without Ca^{+2}. The Ca^{+2}-activated neutral proteinase was determined by measuring the difference in absorbance between assay tubes after incubation with and without Ca^{+2}. Final concentrations of assay reagents were: 50 mM Tris buffer (pH 7.0), 2 mM EDTA (in assay tubes without Ca^{+2}), 2 mg/ml casein-yellow (CalBiochem, La Jolla, California), 1 mM $CaCl_2$ (in assay tubes for Ca^{+2}-stimulated proteinase activity), and 20 mg wet weight of sample from a 10% whol homogenate. Whole homogenate samples were used since preliminary studies showed that approximately 60% of the Ca^{+2}-stimulated neutral proteinase activity was found in the 800 $g_{(max)}$ pellet after centrifugation. The incubation of the assay tubes was performed in a shaking water bath at 37° C for 20 h. The activity of Ca^{+2}-activated proteinase was approximately 46% of the final activity after 10 h of incubation, indicating that the assay approximates linearity over the 20 h of incubation. After incubation the reaction was stopped by the addition of perchloric acid yielding a final concentration of 5%. The precipitates were settled by centrifugation at 1000 g for 10 min and the supernatants were read directly at 295 nm on a Beckman DU-8 spectrophotometer.

Statistical analysis was performed using analysis of variance.

RESULTS

Body weight, muscle weight, and muscle weight to body weight ratios

On the third postburn day, no significant changes in body weight or muscle weight were observed (Table 1). By 21 days after injury, the burned

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17. Kar NC, Pearson CM: A calcium-activated neutral protease in normal and dystrophic human muscle. Clin Chim Acta 73:293-297, 1976.

group of animals had lower (15%) body weights than sham controls and lower soleus weights (12%). However, the soleus weight to body weight ratios were statistically equal between groups, indicating that the decrease in muscle weight was proportional to the decrease in body weight after injury (Table 1).

Proteolytic activity

The soleus had higher rates of neutral proteinase activity (49%) and Ca^{+2}-activated neutral proteinase activity (61%) in comparison to the gastrocnemius muscle (Table 2). The neutral proteinase activity in the absence of Ca^{+2} decreased in the soleus (33%) and gastrocnemius (32%) on the third postburn day (Table 2). By 21 days after injury the neutral proteinase activity in both muscles returned to control values. The Ca^{+2}-activated neutral proteinase activity was not altered in either muscle at 3 or 21 days postburn (Table 2). These results indicate that on the third postburn day, neutral proteinase activity is depressed significantly, but Ca^{+2}-activated neutral proteinase is unaltered in both muscles (Table 2). By 21 days after injury, neutral proteinase activity (without Ca^{+2}) and Ca^{+2}-activated neutral proteinase activity are within control levels (Table 2).

DISCUSSION

Although the role played by intracellular proteinases in protein synthesis and degradation is not clear, alterations in neutral proteinase activity have been observed during skeletal muscle disease, such as muscular dystrophy (17), and during immobilization (18), and denervation (19). Due to their substrate specificity, pH optimum, intracellular localization, and low activity, neutral proteinases are a possible point of regulation for protein degradation.

In this investigation, neutral proteinase activity was measured in the absence and presence of Ca^{+2}. The proteolytic substrate used was casein-yellow, a nitrated casein soluble at physiological pH. These results indicate that neutral proteinases degrade casein-yellow in the absence of Ca^{+2} and degradation is enhanced in the presence of Ca^{+2}. Casein-yellow is one of the few artificial proteolytic substrates that is degraded by a Ca^{+2}-activated neutral proteinase; hemoglobin and myoglobin do not demonstrate enhanced proteolytic degradation in the presence of Ca^{+2} (17). Since Ca^{+2}-activated neutral proteinase activity was of interest in this study because of its ability to degrade troponin protein from

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18. Jakubiec-Puka A, Drabikowski W: Changes in proteolytic activity in muscle of rat after immobilization. In Structure and Function of Normal and Diseased Muscle and Peripheral Nerve, I. Hausmanowa-Petrusewicz and H. Jedrzejowska, eds., Polish Medical Publ., Warsaw, pp 93-97, 1974.

19. Jakubiec-Puka A, Drabikowski W: Influence of denervation and reinnervation of autolytic activity and on protein composition of skeletal muscle in rat. Enzyme 23:10-21, 1978.

myofibrils preferentially (12,13), casein-yellow was employed as the substrate since it can be degraded by Ca^{+2}-activated neutral proteinase.

The observations presented in this study represent results from an *in vitro* assay system. Obviously, the physiological substrate for muscle proteinases is not casein-yellow and the intracellular Ca^{+2} levels do not reach 1 mM *in vivo*. Therefore, these results should not be interpreted literally with regard to the rate of proteolysis *in vivo*. The measured proteinase activities, however, do represent the relative proteolytic capacities between experimental groups.

The results of this investigation indicate that the injured rats had no increase in body weight or muscle weight during the postburn period, while sham-treated control animals gained body weight and muscle weight. The muscle weight to body weight ratios remained constant during the postburn period in both experimental groups, indicating that the changes in muscle weight were proportional to changes in body weight. Since muscle weight fluctuates with injury and disease, it is probable that muscle protein synthesis and/or degradation is altered. Other investigators have shown that excess nitrogen is lost from muscle after injury (15), and these alterations are believed to be due to changes in the rates of protein synthesis and/or degradation (20,21).

These results further show that the soleus has higher levels of neutral proteinase and Ca^{+2}-activated neutral proteinase activity than the gastrocnemius. This could indicate that muscles which derive most of their energy by aerobic means, or are metabolically more active over an extended period of time, require higher levels of proteinases to aid in the degradation of protein from pools which are turning over at faster rates.

Neutral proteinase activity in the absence of Ca^{+2} decreased significantly on the third postburn day in both muscles and returned toward normal by 21 days postinjury. Such neutral proteinase activity is believed to be due to enzyme(s) other than Ca^{+2}-activated neutral proteinase (6), and these enzymes are thought to degrade sarcoplasmic proteins other than myofibrillar protein preferentially (6).

Burn injury causes physiological alterations which preferentially affect these neutral proteinases. The decrease in neutral proteinase activity implies: (1) neutral proteinase enzyme is lost from the sarcoplasm after burn injury, (2) neutral proteinase activity is preferentially inhibited in the burned group, or (3) decreased enzyme synthesis or

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20. Crane CW, Picou D, Smith R, Waterlow JC: Protein turnover in patients before and after elective orthopaedic operations. Br J Surg 64: 129-133, 1977.

21. Fulks RM, Li JB, Goldberg AL: Effects of insulin, glucose, and amino acids on protein turnover in rat diaphragm. J Biol Chem 250:290-298, 1975.

Table 1. Body Weights, Soleus Muscle Weights, and Soleus Muscle Weights to Body Weight Ratios

	Body weight (g)	Soleus weight (mg)	Soleus weight:body weight ratio (mg/g)
3 days postburn			
Sham	450 ± 17	160 ± 18	.35 ± .07
Burned	433 ± 5	158 ± 7	.36 ± .04
21 days postburn			
Sham	499 ± 17	165 ± 5	.33 ± .02
Burned	429 ± 14[a]	148 ± 6[a]	.34 ± .03

Values are $\bar{X}$ ± SEM.

[a] $P < 0.05$ vs. sham.

Table 2. Proteolytic Activity in Rat Skeletal Muscle after Thermal Injury

Group	Neutral proteinase activity (no Ca^{+2})	Neutral proteinase activity (1 mM Ca^{+2})	Ca^{+2}-activated neutral proteinase
Sham controls			
Soleus (7)	.421 ± .018	.957 ± .065	.536 ± .050
Gastrocnemius (7)	.282 ± .008	.593 ± .032	.311 ± .026
3 days postburn			
Soleus (7)	.280 ± .021[a]	.847 ± .073	.567 ± .054
Gastrocnemius (7)	.193 ± .019[a]	.472 ± .043	.279 ± .026
21 days postburn			
Soleus (7)	.390 ± .020	1.048 ± .082	.658 ± .064
Gastrocnemius (7)	.225 ± .016	.507 ± .033	.282 ± .019

Values are $\bar{X}$ ± SEM, N per group in ( ).

[a] $P < 0.05$ vs. sham controls.

Activities represent the change in absorbance of acid extract at 295 nm produced by incubating 20 mg wet wt. of sample at 37° C for 20 h (Abs. units/20 h/20 mg). Proteolytic substrate was casein-yellow.

increased degradation occurs after injury. Perhaps the decrease in proteolytic activity observed at 3 days postinjury is an effort to spare muscle protein during the first few days after injury, prior to an increase in selected enzyme proteins which is observed during the hypermetabolic period after thermal injury.

By 3 days postinjury the rat has recovered from the initial shock associated with thermal injury and is believed to be experiencing alterations in metabolism which eventually cause a state of hypermetabolism by 7-13 days postburn (16). Previous work has shown that selected mitochondrial and sarcoplasmic enzyme levels are elevated in rat muscle during the hypermetabolic period (22). The decrease in neutral proteinase activity on the third day after injury might be one of the initial intracellular alterations prior to the increase in selected muscle enzymes observed during the hypermetabolic period.

This investigation has demonstrated that muscle from thermally injured rats has decreased neutral proteinase activity at 3 days postburn, but activity returns toward normal by 21 days postinjury. The mechanism responsible for this decrease in activity at 3 days postinjury is unknown.

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22. Newman JJ, Strome DR, Goodwin CW, Mason AD Jr, Pruitt BA Jr: Altered muscle metabolism in rats after thermal injury. Metabolism, to be published in December 1982 issue.

PRESENTATIONS

Newman JJ: Altered muscle metabolism in rats following thermal injury. Presented at the Sixty-sixth Annual Meeting of the Federation of American Societies for Experimental Biology, New Orleans, Louisiana, 22 April 1982.

Newman JJ: Altered enzyme activity in rat muscle after thermal injury. Presented at the Fourteenth Annual Meeting of the American Burn Association, Boston, Massachusetts, 13 May 1982.

PUBLICATIONS

Newman JJ, Goodwin CW, Mason AD Jr, Pruitt BA Jr: Altered muscle metabolism in rats following thermal injury. Fed Proc 41:1523, 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0G 6976	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8A. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	H. TERM	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	089
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)* (U) Use of a Laminar Flow Isolator to Control Infection in Burned Troops

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
77 09	82 09	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	0.2	2
FISCAL YEAR CURRENT 83	0.0	0

19. RESPONSIBLE DOD ORGANIZATION
NAME:* US Army Institute of Surgical Research
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RESPONSIBLE INDIVIDUAL
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TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION
NAME:* US Army Institute of Surgical Research Clinical Division
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* William F. McManus, COL, MC
TELEPHONE: 512-221-3301
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME: POC: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procede EACH with Security Classification Code)
(U) Burn Injury; (U) Infection; (U) Laminar Flow; (U) Humans; (U) Wound Colonization

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) It has been well known in recent years that the development of infection has been the most common cause of death in burned soldiers. As the vast majority of these cases result from invasive infection of the burn wound, methods of reducing burn wound contamination would be expected to result in improved survival. In addition, studies have shown that cross-contamination colonization causes more invasive burn wound infections than auto-contamination colonization. These facts generated interest in the use of laminar air flow isolator units as part of burn care.

24. (U) The Sci-Med Company of Minneapolis, Minnesota, was contracted to develop a Laminar air flow unit to meet certain specifications. Following temporary installation and initial patient trials, necessary modifications were undertaken and the unit was redesigned and replaced. Comparison of burn wound colonization between laminar flow and conventionally treated patients is now in progress.

25. (U) 8110 - 8209. Termination Summary. Continued experience with the laminar air flow isolation unit has demonstrated serious technical limitation in the care of severely injured adults and children. These limitations include impairment of ability to monitor the

*Available to contractors upon originator's approval.

DD FORM 1 MAR 88 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

patient, serious sensory deprivation of small children, inability to maintain the curtain barrier, increased difficulty in obtaining laboratory specimens and adequate roentgenographic examinations, and the increase in nursing personnel required to care for the patient in the unit. Moreover increased difficulty in maintaining the laminar flow environment when the patient required ventilatory support was significant limiting feature imposed upon the care of adults and children when placed in the laminar air flow isolator. With these serious limitations and an absence of identifiable benefit, this protocol is terminated.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: USE OF A LAMINAR FLOW ISOLATOR TO CONTROL INFECTION IN BURNED TROOPS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1981 - 30 September 1982

Investigators:

William F. McManus, M.D., Colonel, MC
Robert B. Lindberg, Ph.D.
Judith Fitzpatrick, Captain, ANC
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO: 3A161101A91CC-00, IN HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: USE OF A LAMINAR FLOW ISOLATOR TO CONTROL INFECTION IN BURNED TROOPS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1981 - 30 September 1982

Investigators: William F. McManus, M.D., Colonel, MC
Robert B. Lindberg, Ph.D.
Judith Fitzpatrick, Captain, ANC
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

Continued evaluation of the laminar flow isolator unit during the period of this report established beyond a shadow of a doubt that the technique of laminar flow isolation impeded the care of the extensively burned individual to the point that the physician and nursing staff were adamently opposed to the use of this device. Recurrent problems with laminar flow isolation included decreased visibility of the patient, increased difficulty in measuring vital signs, maintaining accurate intake and output records, caring for the burn wound, turning and mobilizing the patient, caring for the complications of thermal injury and early recognition of subtle changes in the patient's condition was impossible. In addition, the sensory deprivation in the pediatric age group was a severely limiting feature to the point that children would not eat. In addition, early colonization occurred despite the laminar flow isolator both from enteric contamination and with Staphylococcus aureus indicating exogenous contamination which vitiated any value of this technique. The increased difficulty of care of the extensively thermally injured patient as well as the inability to adequately monitor critically burned patients caused us to discontinue the use of the laminar flow isolator since the disadvantages more than clearly exceeded potential benefit. Such techniques may be applicable to patients with a lesser magnitude of injury, however their necessity in such patients would also be questioned.

ABSTRACT

PROJECT NO: 3A161101A91C-00, IN HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: USE OF A LAMINAR FLOW ISOLATOR TO CONTROL INFECTION IN BURNED TROOPS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

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Reports Control Symbol MEDDH-288 (R1)

Continued evaluation of the laminar flow isolator unit during the period of this report established on clinical grounds that the technique of laminar flow isolation impeded the care of the extensively burned individual to the point that the physician and nursing staff were opposed to the use of this device. Recurrent problems with laminar flow isolation included decreased visibility of the patient; increased difficulty in measuring vital signs, maintaining accurate intake and output records, caring for the burn wound, turning and mobilizing the patient, caring for the complications of thermal injury; and impaired ability to recognize subtle changes in the patient's condition. In addition, the sensory deprivation in the pediatric age group was a severely limiting feature to the point that children would not eat. In addition, early colonization occurred despite the laminar flow isolator both from enteric contamination and with Staphylococcus aureus indicating exogenous contamination which vitiated any value of this technique. The increased difficulty of care of the extensively thermally injured patient as well as the inability to adequately monitor critically burned patients caused us to discontinue the use of the laminar flow isolator since the disadvantages more than clearly exceeded potential benefit. Such techniques may be applicable to patients with a lesser magnitude of injury, however their necessity in such patients would also be questioned.

PRESENTATIONS

1 January 1981 - 31 December 1981

Pruitt BA Jr: Current Concepts of Burn Care. Coco Solo Hospital, Panama Canal Zone, 12 January 1981

Pruitt BA Jr: Recent Advances in Burn Care. Medical Assn of the Isthmian Canal Zone, Panama, 12 January 1981

Pruitt BA Jr: Pulmonary Complications of Thermal Injury, Including Inhalation Injury, Gorgas Army Hospital, Panama, 13 January 1981

Pruitt BA Jr: Management of the Burn Wound, Gorgas Army Hospital, Panama, 13 January 1981

Pruitt BA Jr: Early Care of the Extensively Burned Patient. Santo Thomas Hospital, Panama, 14 January 1981

Pruitt BA Jr: Management of Burn Patients in a Combat Environment. Gorgas Army Hospital, Panama, 14 January 1981

Mansour EH: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX, 14 January 1981

Pruitt BA Jr: Metabolic Changes and Nutrition of Burn Patients. Gorgas Army Hospital, Panama, 15 January 1981

Pruitt BA Jr: Burn Care: From Hopelessness to Hope. Evanston Hospital Burn Center, Evanston, IL, 19 January 1981

Pruitt BA Jr: Triage and Initial Care of Burns. Robert B. Green Hospital, San Antonio, TX, 4 February 1981

McManus WF: Classification of Burns. Intensive Care Nurse Clinician Course students, BAMC, Ft Sam Houston, TX, 5 February 1981

McManus WF: Complications of Burns. Intensive Care Nurse Clinician Course students, BAMC, Ft Sam Houston, TX, 5 February 1981

Strieper G: Burn Nursing, Presented to BAMC ICU Course Students, BAMC, Ft Sam Houston, Texas, 6 February 1981

Maguire M: Physical Therapy in Burns. Intensive Care Nurse Clinician Course students, Ft Sam Houston, TX, 9 February 1981

Fullerton J: Role of Occupational Therapy in the Thermally Injured Patient. Intensive Care Nurse Clinician students, Ft Sam Houston, TX, 9 February 1981

Pruitt BA Jr: Care of Burn Patients and Combat Environment Modification Thereof. Brooks Aerospace, San Antonio, TX, 11 February 1981

Pruitt BA Jr: Wound Care and the Diagnosis and Treatment of Inhalation Injury. Brooks Aerospace, San Antonio, TX, 11 February 1981

Cheney V: Burn Nursing, Presented to the Nursing Students of Brackenridge Hospital School of Nursing, Austin, Texas, 11 February 1981

Terry J: Emergency Care in Burns, Presented to the Physicians Assistant Students at AHS, Ft Sam Houston, Texas, 13 February 1981

Cheney V: Overview of Burn Care, Presented to the Association of Critical Care Nurses, San Antonio, Texas, 17 February 1981

McManus WF: Modern Trends in Burn Management. Southwest Missouri Chapter American College of Surgeons. San Antonio, Texas, 20 February 1981

Cheney V: Burn Nursing, Presented to the Nursing Students of the Baptist Hospital School of Nursing, San Antonio, Texas, 23 February 1981

Cheney V: Burn Management, Presented to the Medical Explorers (Boy Scouts), San Antonio, Texas, 25 February 1981

Pruitt BA Jr: Early Care of the Burn Patient - Minor and Major. Wesley Medical Center Trauma - Initial Care Symposium, Wichita, KS, 27 February 1981

Pruitt BA Jr: Life-Threatening Complications of Thermal Injury. Wesley Medical Center Trauma - Initial Care Symposium, Wichita, KS, 27 February 1981

Allen RC: Oxygen-dependent Streptococcus faecalis Chemiluminescence: The Importance of Metabolism and Medium Composition. American Society for Microbiology, 81st Annual Meeting, Dallas, Texas, 1-6 March 1981

McManus AT: Suppression of Pseudomonas aeruginosa Burn Surface Infection by Plasmid RP1. Annual Meeting of the American Society of Microbiology, Dallas, Texas, 2 March 1981

Lindberg RB: Pseudomonas Sepsis in Burned Patients: Its Relationship to Epidemic Septicemia Caused by Three Enteric Species. Annual Meeting of the American Society of Microbiology, Dallas, Texas, 4 March 1981

Maguire M: Physical Therapy and Thermal Injuries. Students 91J School, BAMC, Ft Sam Houston, TX, 4 March 1981

Maguire M: Evaluation of the Upper Extremity in Sports. Medical Explorers (Scouts), San Antonio, Texas, 5 March 1981

Pruitt BA Jr: Early Care of the Severely Burned Patient. USC Critical Care Medicine Course, Las Vegas, NV, 5-7 March 1981

Pruitt BA Jr: Metabolic Alterations Following Multisystemic Injury and Implications of Nutritional Management, USC Critical Care Medicine Course, Las Vegas, NV, 5-7 March 1981

Pruitt BA Jr: Nutritional Management of Severely Injured Patients. USC Critical Care Medicine Course, Las Vegas, NV, 5-7 March 1981

Pruitt BA Jr: Massive Body Burns, USC Critical Care Medicine Course, Las Vegas, NV, 5-7 March 1981

Pruitt BA Jr: Pulmonary Complications Following Massive Body Burn Injuries. USC Critical Care Medicine Course, Las Vegas, NV, 5-7 March 1981

Lawyer R: AORN Conference, Dallas, Texas, 8 - 13 March 1981

Pruitt BA Jr: Initial Assessment of Burn Patients. Brown University, School of Medicine, Department of Surgery, Providence, RI, 12-14 March 1981

McManus WF: Management of the Burn Patient. Army Science Board briefing, Fort Sam Houston, TX, 17 March 1981

Maguire M: P.T. and the Thermally Injured patient, USAF P.T. students, Wilford Hall USAF Medical Center, Lackland AFB, TX, 17 March 1981

Fullerton J: O.T.'s Role with Thermally Injured Patients. Social Work Service, Relatives of Patients. Ft Sam Houston, Texas, 18 March 1981

Pruitt BA Jr: Care of Burn Patients in a Combat Environment. US Army Reserve Medical Symposium, Oklahoma City, OK, 19 March 1981

Maguire M: Care of the Burn Patient. Baylor Univ Master's P.T. students, Academy of Health Sciences, Ft Sam Houston, Texas, 24-25 March 1981

McManus WF: Recent Advances in Burn Care. Oklahoma Surgical Society, Ft Sam Houston, Texas, 26 March 1981

Pruitt BA Jr: Current Techniques of Burn Care. Oklahoma Surgical Society, Ft Sam Houston, Texas, 26 March 1981

Mansour EH: Treatment of Burns. Officer's Basic Course, Academy of Health Sciences, Ft Sam Houston, TX, 27 March 1981

Pruitt BA Jr: Initial Assessment of Burn Patients, Ft Sam Houston Advanced Trauma Life Support, Ft Sam Houston, TX, 29 March 1981

Cheney V: Overview of Burns, Presented to the Nursing Educators at BAMC, Ft Sam Houston, Texas, 31 March 1981

Pruitt BA Jr: Stress Ulcers and Postinjury Pancreatitis. ACS Spring Meeting, New Orleans, LA, 1 April 1981

Kim SH: An Evaluation of Burn Wound Biopsy. 13th Annual Meeting of the American Burn Association, Washington, DC, 2-4 April 1981

Pruitt BA Jr: Planning, Implementing and Evaluating the Importance of Public Education Programs. American Burn Assn Annual Meeting, Washington, D.C., 3 April 1981

Becker RA: A Syndrome of Secondary or Tertiary Hypothyroidism in Septic, Terminally Ill Burn Patients. Annual Meeting of the American Burn Association, Washington, DC, 4 April 1981

Maguire M: The Evaluation of the Lower Leg and Overuse Syndromes. HSC Musculoskeletal Course, Ft Sam Houston, TX, 8 April 1981

Maguire M: Evaluation and Treatment of the Elbow, Wrist and Hand. HSC Musculoskeletal Course, Ft Sam Houston, TX, 14 April 1981

Maguire M: Evaluation of the Hip and Its Treatment. HSC Musculoskeletal Course, Ft Sam Houston, TX, 15 April 1981

Powanda MC: Indices of Infection and/or Inflammation in the Burned and Burned-infected Rat. Annual Meeting of the Federation of American Societies for Experimental Biology, Atlanta, Georgia, 15 April 1981

Pruitt BA Jr: Initial Care of the Burn Patient. Wilford Hall USAF Medical Center, Lackland AFB, TX, 16-18 April 1981

Fullerton J: The Role of the Occupational Therapist in the Care of the Burn Patient. Occupational Therapy students, St. Phillip's College, San Antonio, TX, 20 April 1981

Allen RC: The Humoral-Phagocyte Axis of Immunity, Department of Immunology, Rush Medical Center, Chicago, Illinois, 23 April 1981

Allen RC: Chemilumigenic Probing: An Approach to Assessment of The Humoral-Phagocyte Axis in the Thermal Injury Patient, Surgical Infection Society, 1st Annual Meeting, Chicago, Illinois, 24-25 April 1981

McManus AT: Experimental Proteus mirabilis Burn Surface Infection. Annual Meeting of the Surgical Infection Society, Chicago, Illinois, 25 April 1981

Pruitt BA Jr: Early Care of the Burn Patient, Brooks AFB Battlefield Medicine Course, San Antonio, TX, 29 April 1981

Pruitt BA Jr: Diagnosis and Treatment of Inhalation Injury: Triage and Aeromedical Transfer of Burn Patients. Brooks AFB Battlefield Medicine Course, San Antonio, TX, 29 April 1981

Syby C: Burn Care, Presented to Incarnate Word School of Nursing, 30 April 1981

Pruitt BA Jr: The ISR Experience with Patients Sustaining Burns in Vietnam. Gary Wratten Symposium, San Antonio, TX, 1 May 1981

Pruitt BA Jr: Current Military Research in Burn Care. 121st ARCOM Medical Seminar, Birmingham, AL, 2 May 1981

Pruitt BA Jr: Gastrointestinal Complications of Injury. El Paso Surgical Society, El Paso, TX, 4 May 1981

Pruitt BA Jr: The Metabolic Response to Injury. Texas Tech Regional Academic Health Center at El Paso, El Paso, TX, 4 May 81

Cheney V: Burn Care, Presented to LVN School, Jourdanton, Texas, 12 May 1981

Pruitt BA Jr: Inhalation Injury to Include Carbon Monoxide Poisoning. Barnes Hospital, St. Louis, MO, 13 May 1981

Pruitt BA Jr: The Metabolic Response to Severe Injury. Barnes Hospital, St. Louis, MO, 13 May 1981

Pruitt BA Jr: Fluid Replacement Following Injury. Barnes Hospital, St. Louis, MO, 13 May 1981

Pruitt Ba Jr: The Diagnosis and Treatment of Opportunistic Infections. Barnes Hospital, St. Louis, MO, 13 May 1981

Strieper G: Burn Care in Disasters, Presented at the University of Utah, Salt Lake City, Utah, 14-15 May 1981

McManus WF: Burns. Nursing Inservice Program, Ft Sam Houston, Texas, 20 May 1981

Cheney V: Burn Update, Presented to Social Work Service, BAMC, Ft Sam Houston, Texas, 27 May 1981

McManus WF: The Mission and Function of the Institute of Surgical Research. Rotary Club, San Antonio, TX, 29 May 1981

McManus WF: Burn Mass Casualty Management. Presented to Second World Congress on Emergency and Disaster Medicine, Pittsburg, PA, 2 June 1981

Pruitt BA Jr: The Diagnosis and Treatment of Burn Wound Infections. Robert Packer Hospital, Sayre, PA, 3-5 June 1981

Cheney V: Burn Nursing, Presented to the Brackenridge School of Nursing, Austin, Texas, 8 June 1981

Cheney V: Overview of Burn Care, Presented to the Recruiting Command, BAMC, Ft Sam Houston, Texas, 9 June 1981

Brown JR: Occupational Therapists' Role with Thermally Injured Patients. Social Work Services, Patient's relatives, Ft Sam Houston, Texas, 10 June 1981

Cheney V: Burns As an Emergency, Presented to the Aviators of Academy of Health Sciences, Ft Sam Houston, TX, 12 June 1981

Pruitt BA Jr: Transportation of Burn Patients. Trauma Symposium, Cleveland, OH, 12-13 June 1981

Pruitt BA Jr: Resuscitation of Burns. Trauma Symposium, Cleveland, OH, 12-13 June 1981

Cheney V: Burn Nursing, Presented at the University of Texas School of Nursing, San Antonio, Texas, 17 June 1981

Vaughan GM: Neurological Basis of Human Melatonin and Cortisol Rhythms. Meeting of Military Endocrinologists, Cincinnati, Ohio, 17 June 1981

Pruitt BA Jr: Diagnosis and Treatment of Inhalation Injury. University of Minnesota Twin Cities, Minneapolis, MN, 18-20 June 1981

Pruitt BA Jr: The Metabolic Response and Nutritional Support of the Burn Patient. University of Minnesota Twin Cities, Minneapolis, MN, 18-20 June 1981

Vaughan GM: Comparison of Human Melatonin and Cortisol Rhythms. Annual Meeting of the Endocrine Society, Cincinnati, Ohio, 19 June 1981

Pruitt BA Jr: Fluid Resuscitation and Metabolic Changes in Burn Patients. Literature Conference UTMC, San Antonio, Texas, 24 June 1981

McManus WF: Electric Injury. Nursing Inservice Program, Ft Sam Houston, Texas, 24 June 1981

Allie J: Physical Therapy and the Burn Patient. 91J students, Academy of Health Sciences, Ft Sam Houston, Texas, 26 June 1981

Cheney V: Overview of Burns, Presented to the Social Work Service, BAMC, Ft Sam Houston, Texas, 22 July 1981

Brown JR: The Role of Occupational Therapists with the Thermally Injured. 91L students, Academy of Health Sciences, Ft Sam Houston, Texas, 22 July 1981

Maguire M: Evaluation of Hip and Upper Extremity in Sports. University of Texas P.T. students, San Antonio, Texas, 24 July 1981

Pruitt BA Jr: Overview of Current Research and Development in the Treatment of Burn Injury. Joint United Kingdom/USN Workshop on Research and Development for Improved Combat Casualty Care, Alverstoke, Hampshire, England, 27-31 July 1981

Maguire M: Evaluation of Hip and Upper Extremity in Sports. University of Texas P.T. students, San Antonio, Texas, 31 July 1981

Lindberg RB: Epidemics of Enterobacteriaceae in Burn Patient Infections. Annual Meeting of the South African Burn Association, Skukuza, South Africa, 5 August 1981

Powanda MC: The Role of Leukocyte Endogenous Mediator (Endogenous Pyrogen) in Inflammation. Symposium: The Roles of Copper and Other Essential Metals in Inflammatory Diseases, College of Pharmacy, University of Arkansas, Little Rock, Arkansas, 10 August 1981

McManus WF: The Mission and Function of the Institute of Surgical Research. Rotary Club, San Antonio, TX, 10 August 1981

Cheney V: Burn Care, Presented to the OR Nursing Course, BAMC, Ft Sam Houston, Texas, 11 August 1981

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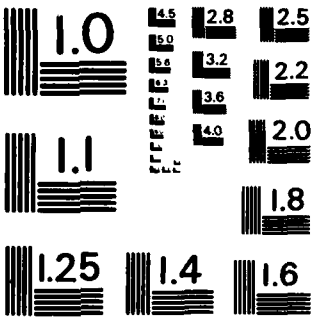

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Pruitt BA Jr: Burn Care in the Emergency Room. Third Annual USAF PA Seminar, San Antonio, Texas, 11 August 1981

Lawyer R: Skin Grafting, Presented to the OR Nursing Course, BAMC, Ft Sam Houston, Texas, 11 August 1981

McManus WF: Classification of Burns. Intensive Care Nurse Clinician Course students, BAMC, Ft Sam Houston, TX, 12 August 1981

Pruitt BA Jr: Current Status of Biologic Dressing. Literature Conference UTMC, San Antonio, Texas, 12 August 1981

McManus WF: Complications of Burns. Intensive Care Nurse Clinician Course students, BAMC, Ft Sam Houston, TX, 13 August 1981

Strieper G: Care of the Thermally Injured, Presented to the ICU Course, BAMC, Ft Sam Houston, Texas, 14 August 1981

Pruitt BA Jr: Hemodynamic Consequences of Burn Injury. Royal Brisbane Hospital Surgery Grand Rounds, Brisbane, Australia, 17 August 1981

Allie J: Physical Therapy and the Burn Patient. Intensive Care Nurse Clinician Course students, BAMC, Ft Sam Houston, Texas, 18 August 1981

Brown JR: Occupational Therapy and the Burn Patient. Intensive Care Nurse Clinician Course students, BAMC, Ft Sam Houston, Texas, 18 August 1981

Pruitt BA Jr: Metabolic Changes After Severe Injury. Royal Australasian College of Surgeons, Dunedin, New Zealand, 18-21 August 1981

Pruitt BA Jr: Current Status of Burn Injury. Royal Australasian College of Surgeons, Dunedin, New Zealand, 18-21 August 1981

Cheney V: Overview of Burns, Presented to the Newly Assigned ANC Officers, BAMC, Ft Sam Houston, TX, 31 August 1981

Maguire M: Physical Therapy and the Burn Patient. Presented at the course entitled O.T. and P.T. Care in the Thermally Injured Patient. Academy of Health Sciences, Ft Sam Houston, Texas, 31 August - 4 September 1981

Brown JR: Occupational Therapy and the Burn Patient. Presented at the course entitled O.T. and P.T. Care in the Thermally Injured Patient. Academy of Health Sciences, Ft Sam Houston, Texas, 31 August - 4 September 1981

Cheney V: Overview of Burns, Presented to the AHSC Officers Workshop, BAMC, Ft Sam Houston, TX, 1 September 1981

Allie J: Physical Therapy and the Burn Patient. Social Work Service, Patient's relatives, Ft Sam Houston, TX, 8 September 1981

McManus WF: Treatment of Burns. Officer's Basic Course, Academy of Health Sciences, Fort Sam Houston, TX, 15 September 1981

Cheney V: Burn Care, Presented to the Social Work Service, BAMC, Ft Sam Houston, Texas, 16 September 1981

Allie J: Physical Therapy and the Burn Patient. USAF P.T.'s Wilford Hall USAF Medical Center, Lackland AFB, Texas, 16 September 1981

Vaughan GM: Cortisol and Corticotrophin After Burn Injury. Annual Meeting of the American Association for the Surgery of Trauma, Hot Springs, Virginia, 17 September 1981

Strieper G: Pathophysiology of Burns and Burn Care, Presented to the Graduate Nursing Students of University of Texas, San Antonio, Texas, 18 September 1981

Goodwin CW: Increased Incidence of Pancreatitis in Thermally Injured Patients: A Prospective Study. Annual Meeting of the American Association for the Surgery of Trauma, Hot Springs, Virginia, 19 September 1981

Fullerton J: Occupational Therapy and the Burn Patient. Health Careers Class, Highlands High School, San Antonio, Texas, 22 September 1981

Pruitt BA Jr: Early Care of Burn Patients. Battlefield Medicine Course, Brooks AFB, TX, 23 September 1981

Pruitt BA Jr: Burn Wound Care and Complications of Thermal Injury. Battlefield Medicine Course, Brooks AFb, TX, 23 September 1981

Pruitt BA Jr: Current Status of Burn Care in the United States. San Antonio Shriners' Meeting, San Antonio, Texas, 23 September 1981

Strieper G: Burn Care as Part of Operational Readiness, Presented to Navy Nurses, National Naval Medical Center, Bethesda, MD, 24 September 1981

McLaurin NK: Nutrition Support of the Critically Ill Patient Using a Continuous Computer Graphic Program (Poster Session), 64th American Dietetic Association Meeting, Philadelphia, Pa, 24 September 1981

Pruitt BA Jr: Current Status of Biologic Dressings. General Motors Surgical Research Conference, Sloan-Kettering, New York, NY, 28 September 1981

Cheney V: Burn Care, Presented to the Nursing Students, University of Texas, San Antonio, TX, 29 September 1981

Stallings RJ: The Burn Patient. Social Service, BAMC, Ft Sam Houston, Texas, 30 September 1981

Allen RC: Chemiluminescence as a Tool for Detection of Dioxygenations. (Invited Presentation) International Conference on "Peroxides in Biological Systems" under the auspices of the European Society for Biochemical Pharmacology. Otzenhausen/Saar, Federal Republic of Germany, September 1981

Allen RC: Direct Quantification of Phagocyte Oxygenation Activity in Whole Blood: A Chemiluminigenic Probe Approach. (Invited Presentation Symposium 21 Luminescence). XI International Congress of Clinical Chemistry and IV European Congress of Clinical Chemistry, Vienna, Austria, September 1981

Allen RC: Native and Probe-Amplified Phagocyte Luminescence, Department of Biology & Microbiology, University of Texas, Austin, Texas, September 1981

McManus WF: Inhalation Injury. Nursing Inservice Program, Ft Sam Houston, Texas, 1 October 1981

Cheney V: Burn Nursing, Presented to Physician's Assistants, AHS, Ft Sam Houston, Texas, 2 October 1981

Cheney V: Burn Nursing, Presented to LVN Association, San Antonio, Texas, 6 October 1981

Pruitt BA Jr: Fluid Management of the Extensively Burned Patient. General Surgery Service, BAMC, Ft Sam Houston, Texas, 6 October 1981

Brown JR: O.T.'s Role with the Thermally Injured. 91L students, Academy of Health Sciences, Ft Sam Houston, Texas, 13 October 1981

Powanda MC: Plasma and Cellular Elements of Blood Interact in the Generation of Biochemical Indicators in Burned Rats. Annual Meeting of the Reticuloendothelial Society, Milwaukee, Wisconsin, 15 October 1981

McManus AT: Examination of Particulate Glucan Effects on Experimental Pseudomonas Burn Wound Sepsis. Annual Meeting of the Reticuloendothelial Society, Milwaukee, Wisconsin, 15 October 1981

Strieper G: Burn Treatment in the NBC Environment, Presented to the 21st General Hospital, St Louis, MO, 18 October 1981

McManus WF: Treatment of Burn Patients. American Medical Record Association, San Antonio, Texas, 21 October 1981

Cheney V: Burns as an Emergency, Presented to the Aviators of AHS, Ft Sam Houston, Texas, 26 October 1981

Pruitt BA Jr: Current Management of Burn Injury. Wilford Hall Surgical Staff Lecture Series, Lackland AFB, Texas, 27 October 1981

Strieper G: Overview of Burn Nursing, Presented to the University of New Mexico, Albuquerque, NM, 27-29 October 1981

Pruitt BA Jr: Epidemiology Triage and Transport of the Burn Patient. Presented at the Annual Meeting of the Association of Military Surgeons of the US, San Antonio, Texas, 2 November 1981

McManus WF: Resuscitation and Early Care of the Burn Patient. Presented at the Annual Meeting of the Association of Military Surgeons of the US, San Antonio, Texas, 2 November 1981

Goodwin CW: Diagnosis and Treatment of Inhalation Injury. Presented at the Annual Meeting of the Association of Military Surgeons of the US, San Antonio, Texas, 2 November 1981

Stallings RJ: Diagnosis, Treatment and Prevention of Burn Wound Infections. Presented at the Annual Meeting of the Association of Military Surgeons of the US, San Antonio, Texas, 2 November 1981

Shirani KZ: Burn Wound Closure Including Excision, Presented at the Annual Meeting of the Association of Military Surgeons of the US, San Antonio, Texas, 2 November 1981

Cheney V: Burn Nursing in Disaster, Presented to the Air Force Nurses, Wilford Hall Medical Center, San Antonio, Texas, 2-4 November 1981

Yurt RW: Treatment of Burns. Officer's Basic Course, Academy of Health Sciences, Ft Sam Houston, TX, 3 November 1981

Allie J: Physical Therapy and the Burn Patient. Social Work Service, patients families, Ft Sam Houston, TX, 4 November 1981

Pruitt BA Jr: Outpatient Management of Minor Burns. Presenting Emergencies Series, Georgia Hospital Association, Atlanta, Georgia, 4 November 1981

Pruitt BA Jr: Metabolic and Nutritional Consequences of Thermal Injury. Bristol Myers Symposium on Nutritional Research. Washington, D.C., 9 November 1981

McManus WF: Traumatic Injury. Clinical Pastoral Chaplain's Course, BAMC, Ft Sam Houston, Texas, 10 November 1981

Brown JR: Occupational Therapy and the Burn Patient. Social Work Service, Patients' relatives, Ft Sam Houston, Texas, 11 November 1981

Cheney V: Overview of Burns, Presented to the Department of Human Resources, San Antonio, Texas, 20 November 1981

Pruitt BA Jr: Modern Burn Therapy. New Jersey Medical School, Newark, NJ, 22-24 November 1981

McManus WF: Care of the Wounds. Nursing Inservice Program. Ft Sam Houston, Texas, 25 November 1981

Strieper G: Management of Burn Patients, Missouri State University, Kirksville, MO, 30 November 1981

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Pruitt BA Jr: Fluid Resuscitation and Metabolic Changes in the Injured Man. Philippine College of Surgeons, Manila, Philippines, 7-11 December 1981

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McManus WF: Lasers and Radiation Burns. Academy of Health Sciences, Ft Sam Houston, Texas, 8 December 1981

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